Atty. Dkt. No. 054707-1276

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Joseph P. Steiner *et al.*

Title: ROTAMASE ENZYME ACTIVITY INHIBITORS

Appl. No.: 09/805,249

Filing Date: 03/14/2001

Examiner: Vickie Y. Kim

Art Unit: 1614

SUGGESTION UNDER 37 C.F.R. § 41.202
THAT AN INTERFERENCE BE DECLARED

Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

Sir:

Applicants suggest under 37 C.F.R. § 41.202(a) that the Office declare an interference between this application and U.S. Patent No. 6,037,370 (the '370 patent) (Exhibit A).

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Exhibit F	U.S. Patent Application No. 08/693,003
Exhibit G	U.S. Patent Application No. 08/479,436 (June 7, 1995)
Exhibit H	U.S. Patent Application No. 08/551,026

A. THE REQUIREMENTS OF 37 C.F.R. § 41.202(a) ARE MET

Section 41.202(a) states as follows:

> **§ 41.202 Suggesting an interference.**
>
> (a) *Applicant.* An applicant, including a reissue applicant, may suggest an interference with another application or a patent. The suggestion must:
>
> (1) Provide sufficient information to identify the application or patent with which the applicant seeks an interference,
>
> (2) Identify all claims the applicant believes interfere, propose one or more counts, and show how the claims correspond to one or more counts,
>
> (3) For each count, provide a claim chart comparing at least one claim of each party corresponding to the count and show why the claims interfere within the meaning of § 41.203(a),
>
> (4) Explain in detail why the applicant will prevail on priority,
>
> (5) If a claim has been added or amended to provoke an interference, provide a claim chart showing the written description for each claim in the applicant's specification, and
>
> (6) For each constructive reduction to practice for which the applicant wishes to be accorded benefit, provide a chart showing where the disclosure provides a constructive reduction to practice within the scope of the interfering subject matter.

37 C.F.R. § 41.202(a). As set forth below, all requirements of § 41.202(a) are met in the present case. The Office should therefore declare an interference as suggested.

1. Sub-Section 41.202(a)(1)

Applicants identify as the patent with which Applicants seek an interference U.S. Patent No. 6,037,370 filed on June 8, 1995, assigned on its face to Vertex Pharmaceuticals Incorporated and naming David M. Armistead as inventor.

2. Sub-Section 41.202(a)(2)

This sub-section requires identifying interfering claims, proposing a count, and explaining how the claims correspond to the count.

a. Identification of interfering claims

Applicants identify claim 6 of the '370 patent as interfering.

b. Proposed count

Applicant's proposed count is:

COUNT: Claim 41 of the present application or claim 6 of the '370 patent

Exhibit B provides the proposed count with the full text of claims 41 and 6. The proposed count is an alternative style count, defined as independent claim 41 of the present application "or" independent claim 6 of the '370 patent.

c. Explanation of how claims correspond to count

Claim 6 of the '370 patent and claim 41 of the present application correspond to the count, because the count recites each in the alternative.

3. Sub-Section 41.202(a)(3)

Applicants provide Exhibit C comparing claims corresponding to the count (claim 41 of the present application to claim 6 of the '370 patent). The claims extensively overlap.

Section 41.203(a) sets forth a two-way test to determine whether an interference exists: "An interference exists if the subject matter of a claim of one party would, if prior art, have anticipated or rendered obvious the subject matter of a claim of the opposing party and vice versa." 37 C.F.R. § 41.203(a).

The comparison set forth in Exhibit C demonstrates that the two-way test for patentability is satisfied in this case. The extensive overlap between these claims shows that the invention as defined by claim 6[1] of the '370 patent anticipates Applicants' invention as defined by claim 41. In turn, Applicants' invention as defined by claim 41 anticipates the '370 patent's invention as defined by claim 6. An interference-in-fact therefore exists between these claims.

Although some language of pending claim 41 differs from that of claim 6 of the '370 patent, one of ordinary skill in the relevant art would readily understand the difference to be purely semantic. For example, pending claim 41 of the present application recites the step of "administering to said nerve cell an effective amount," while the first step of claim 6 of the '370 patent is "contacting said nerve cells with a composition comprising a neurotrophic amount." The skilled artisan would understand that the meaning of these two phrases is identical since "an effective amount" of a compound for stimulating neurite outgrowth (pending claim 41) is a neurotrophic amount ('370 patent claim 6) of that compound. For example, Examples 2 and 3 of the '370 patent (see columns 23-24) disclose that the neurotrophic activity of the FKBP12 binding compounds utilized in the invention may be determined by measuring neurite outgrowth in PC12 cells or dorsal root ganglion cell cultures.

It is readily apparent -- from the side-by-side comparison in Exhibit C of claim 41 of the present application and claim 6 of the '370 patent -- that these claims satisfy the two-way test of § 41.203(a). Therefore, an interference exists.

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[1] A proviso in claim 6 of the '370 patent excludes a relatively small subgenus of compounds and only minimally affects the extensive overlap with claim 41 of the present application.

The USPTO should therefore declare an interference between the present application and the '370 patent and, in particular, between the invention of pending claim 41 and claim 6 of the '370 patent.

4. Sub-Section 41.202(a)(4)

Section 41.202(a)(4) requires a detailed explanation of why Applicants will prevail.

Applicants will prevail because the application deserves an earlier accorded benefit for the count. 37 C.F.R. § 41.207(a). Applicants would be senior party because the present application and its priority applications provide a constructive reduction to practice before June 8, 1995, the earliest possible effective filing date of the '370 patent. This is a sufficient showing of priority that, if unrebutted, would support a determination of priority in favor of Applicants. 37 C.F.R. § 41.202(e).

The '370 patent issued from U.S. Application No. 08/486,004, filed June 8, 1995. The '370 patent claims priority to no earlier applications. The earliest possible effective filing date to which the '370 patent may be entitled is thus June 8, 1995.[2]

As explained in detail below, the present application benefits from a priority date one day earlier: June 7, 1995. The present application thus benefits from an effective filing date earlier than the filing date of the '370 patent. Specifically, two species of the present application benefit from the filing date of application 08/479,436 under 35 U.S.C. § 120 because there is continuity of disclosure, copendency, and inventorship.

[2] Applicants do not concede that the '370 patent claims are entitled to the benefit of the underlying application's filing date of June 8, 1995.

a. Constructive reduction to practice on June 7, 1995

A constructive reduction to practice is "a described and enabled anticipation . . . in a patent application . . . of a count" and the earliest constructive reduction to practice is "the first constructive reduction to practice that has been continuously disclosed through a chain of patent applications including in the involved application or patent." 37 C.F.R. § 41.201.

The claim chart of Exhibit D demonstrates that Applicants' earliest-filed application 08/479,436 (Exhibit G), filed June 7, 1995, is a benefit application providing constructive reduction to practice of at least two embodiments within the scope of the proposed count.

The present application is a:

- Continuation-in-part of 08/551,026, filed October 31, 1995 (Exhibit H[3])
- Continuation-in-part of 09/359,351, filed July 21, 1999 (Exhibit E)
  - which is a continuation of 08/693,003, filed August 6, 1996 (Exhibit F)
    - which is a continuation of 08/479,436 (Exhibit G), filed June 7, 1995, now U.S. Patent No. 5,614,547

Identical disclosures appear in applications 09/359,351; 08/693,003; and 08/479,436 (collectively the priority applications).

The '436 application discloses the method recited by the proposed count. The '436 application states: "[t]he neurotrophic compounds of this invention can be periodically administered to a patient undergoing treatment for neurological disorders or for other reasons in which it is desirable to stimulate neuronal regeneration and growth . . . ." (Exhibit G, page 21, lines 10-14). One of ordinary skill in the art would readily understand this phrase to disclose "[a] method for stimulating neurite outgrowth by a nerve cell . . ." recited by the

[3] Included for completeness but not necessary for the requested accorded benefit.

proposed count, because stimulating neurite outgrowth would be the goal of administering a neurotrophic compound to such a patient.

Exhibit D (claim chart) shows how Species 1 and 2 of the ultimate parent application 08/479,436 establish constructive reduction to practice of the proposed count. One of ordinary skill in the art would recognize that Species 1 and 2 are encompassed by Formula I of the count. The '436 application (Exhibit G) discloses and describes how to make Species 1 in Example 10 (page 38). The '436 application (Exhibit G) discloses Species 2 (page 19, lines 19-20) and describes how to make Species 2 in the general disclosure of Example 4 (page 34). The '436 application thus enables one skilled in the art to make Species 1 and 2.

Finally, the '436 application enables one skilled in the art to use Species 1 and 2 to stimulate neurite outgrowth. Table II (Exhibit G, page 30), for example, demonstrates how to use Species 1 and 2 by disclosing appropriate doses of these compounds for stimulating neurite outgrowth.

b. There is continuity of disclosure, pendency, inventorship

Continuity of disclosure exists. The three relevant priority applications (09/359,351; 08/693,003; and 08/479,436) are identical. The following table identifies relevant disclosures in the present and priority applications.

Disclosure	**Present Application** 09/805,249	**Priority Applications** 09/359,351 (Exhibit E) 08/693,003 (Exhibit F) 08/479,436 (Exhibit G)
Treating neurological disorders	Page 13, lines 11-13	Page 21, lines 10-14
Administration	Page 14, line 7, to page 17, line 4	Page 22, line 11, to page 26, line 4, and Table II (page 30)
Species 1	Example 11 (page 29)	Example 10 (page 38)
Species 2	Example 17 (page 32)	Page 19, lines 19-20. General preparation in Example 4 (page 34)

Continuity of pendency exists. The table below presents the relevant filing, issuance, and abandonment dates to demonstrate continuity of pendency.

Application No.	**Patent No.**	**Filed**	**Issued/Abandoned**
09/805,249	Pending	**3/14/2001**	———
09/359,351 (CONT of '003)	6,509,477	**7/21/1999**	**1/21/2003**
08/693,003 (CONT of '436)	Abandoned	**8/6/1996**	**9/9/1999**
08/479,436	5,614,547	**6/7/1995**	**3/25/1997**

Continuity of inventorship exists. The inventive entity is identical for the present application and the priority applications: Gregory Hamilton and Joseph P. Steiner.

Consequently, the present application should be accorded benefit of the filing date of June 7, 1995, for priority purposes. 37 C.F.R. § 41.201. Given that benefit, Applicants should be designated as the senior party in the interference. 37 C.F.R. 41.207.

5. Sub-Section 41.202(a)(5)

Section 41.202(a)(5) does not apply because the application contains no new claims.

Of the pending claims, only claims 41 and 44 have been amended from their originally-filed text, but those amendments did not introduce new claims. Applicants have identified at least claim 41 as corresponding to the count. Claim 41 as amended corresponds to claim 43 as originally filed. Claim 41 as originally filed recited that J and K together form a 5-7 membered heterocyclic ring. Claim 43 as originally filed recited "The method of claim 41 wherein J and K are taken together to form a 5 membered heterocyclic ring." On June 13, 2003, Applicants canceled claim 43 and amended Claim 41 to incorporate the limitations of claim 43 by replacing "5-7 membered heterocyclic ring" in the definition of J and K in claim 41 with "5 membered heterocyclic ring." This amendment had the same effect as simply rewriting claim 43 in independent form. Claim 44, which originally depended from canceled claim 43, was amended to depend from claim 41.

Applicants have presented no other amendments to the currently pending claims of the present application.

6. Sub-Section 41.202(a)(6)

Exhibit D is a chart showing disclosures confirming constructive reduction to practice of two species of claim 41 of the present application Specifically, the chart shows Species 1[4] and Species 2[5] and related disclosures in the priority applications.

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[4] See the present application, Example 11, p. 29; priority applications, Example 10, p. 38, l. 13.

[5] 1,7-diphenyl-4-heptyl (2*S*)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate. See the present application, Example 17, p. 32; priority applications, p. 19, ll. 19-20, and Example 4, p. 34.

B. MISCELLANEOUS

All claim presently pending in this application are in condition for allowance upon entry of the Reply to the outstanding Office Action filed concurrently herewith. At least claim 41 corresponds to the proposed count, as explained in detail above.

1. The Requirements Of 35 U.S.C. § 135(b) Are Met

Section 135(b) precludes an Applicant from adding a claim that is the same or substantially the same subject matter as the claims of an issued patent over one year after that patent issued. 35 U.S.C. § 135(b)(1).

The '370 patent issued on March 14, 2000. On March 14, 2001, less than one year after the '370 patent issued, Applicants filed the present application.[6] As explained in subsection 5 above, Applicants have added no new claims since then because the currently pending claims are identical to claims in the present application as originally filed.

2. The Application And The '370 Patent Are Not Commonly Owned

An administrative patent judge may decline to declare an interference between commonly-owned applications and patents. 37 C.F.R. § 41.206.

Here, the present application and the '370 patent are not commonly-owned. They are assigned to independent entities. The present application is assigned to GPI NIL Holdings, Inc. (assignment recorded at Reel 12281, Frame 0063, on October 23, 2001). The '370 patent

[6] "Note that the expression 'prior to one year from the date on which the patent was granted' in 35 U.S.C. 135(b) includes the one-year anniversary date of the issuance of a patent." M.P.E.P. 2307 (8th ed., Rev. 2, May 2004) (citing *Switzer v. Sockman*, 333 F.2d 935, 142 USPQ 226 (CCPA 1964).

is assigned to Vertex Pharmaceuticals Corporation (assignment recorded at Reel 007891, Frame 0912, on April 15, 1996).

3. The '370 Patent Is Unexpired And No Maintenance Fees Are Due

According to the USPTO Patent Application Information Retrieval system, no maintenance fees are currently due in the '370 patent, and the next payment window opens March 14, 2007. The '370 patent is thus unexpired and qualifies for declaration of interference. *See* M.P.E.P. 2309, 35 U.S.C. § 135(a).

C. CONCLUSION

Accordingly, Applicants respectfully suggest that the USPTO (1) declare an interference between the present application and the '370 patent with the proposed count, (2) designate as corresponding to the count at least claim 6 of the '370 patent, and (3) declare Applicants the senior party based on accorded benefit of the June 7, 1995, filing date of application 08/479,436.

If any fees are due in connection with the filing of this Request for Interference, please charge such fees (or credit overpayment) to Deposit Account No. 19-0741.

Respectfully submitted,

Date 10/6/2004

By _____

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EXHIBIT B

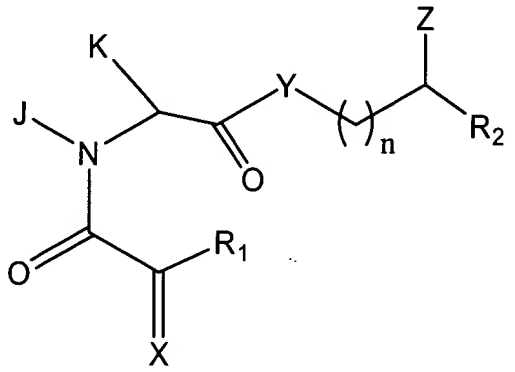
PROPOSED COUNT:

(Claim 41 of 09/805,249 OR Claim 6 of 6,037,370)

Claim 41 of Application No. 09/805,249

A method for stimulating neurite outgrowth by a nerve cell, comprising:

administering to said nerve cell an effective amount of compound having an affinity for FKBP-type immunophilins according to formula I



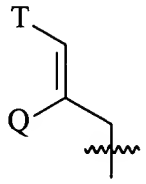
Formula I

or a pharmaceutically acceptable salt thereof,

- wherein Y is CH_2, O, NH, or N-(C1-C4 alkyl);

- wherein Z and R_2 are independently Ar, (C5-C7)-cycloalkyl substituted (C1-C6)-straight or branched alkyl or alkenyl, (C5-C7)-cycloalkenyl substituted (C1-C6)-straight or branched alkyl or alkenyl, or Ar substituted (C1-C6)-straight or branched alkyl or alkenyl, wherein in each case, one or two carbon atoms of the straight or branched alkyl or alkenyl groups may be substituted with 1-2 heteroatoms selected from the group

consisting of oxygen, sulfur, SO and SO_2 in chemically reasonable substitution patterns, or



- wherein Q is hydrogen, (C1-C6)-straight or branched alkyl or (C1-C6) - straight or branched alkenyl;

- wherein T is Ar or substituted 5-7 membered cycloalkyl with substituents at positions 3 and 4 which are independently selected from the group consisting of hydrogen, hydroxyl, O-(C1-C4)-alkyl or O-(C1-C4)-alkenyl and carbonyl;

- wherein Ar is selected from the group consisting of monocyclic and bicyclic heterocyclic aromatic ring systems with individual ring sizes being 5 or 6, which may contain in either or both rings a total of 1-4 heteroatoms independently selected from oxygen, nitrogen and sulfur; wherein Ar may contain one to three substituents which are independently selected from the group consisting of hydrogen, halo, hydroxyl, hydroxymethyl, nitro, CF_3, trifluoromethoxy, (C1-C6)-straight or branched alkyl or (C1-C6)-straight or branched alkenyl, O-(C1-C4)-straight or branched alkyl or O-(C1-C4)-straight or branched alkenyl, O-benzyl, O-phenyl, amino, 1,2-methylenedioxy, carbonyl and phenyl;

- wherein R_1 is either hydrogen or U; X is either oxygen or CH-U, provided that if R_1 is hydrogen, then X is CH-U, or if X is oxygen then R_1 is U;

- wherein U is hydrogen, O-(C1-C4)-straight or branched alkyl or O-(C1-C4)-straight or branched alkenyl, (C1-C6)-straight or branched alkyl or (C1-C6)-straight or branched alkenyl, (C5-C7)-cycloalkyl, (C5-C7)-cycloalkenyl substituted with (C1-C4)-straight or branched alkyl or (C1-C4)-straight or branched alkenyl, [(C1-C4)-alkyl or (C1-C4)-alkenyl]-Ar or Ar (Ar as described above);

- wherein J is hydrogen or C1 or C2 alkyl or benzyl; K is (C1-C4)-straight or branched alkyl, benzyl or cyclohexylethyl; or wherein J and K may be taken together to form a 5 membered heterocyclic ring which may contain an oxygen (O), sulfur (S), SO or SO_2 substituted therein; and

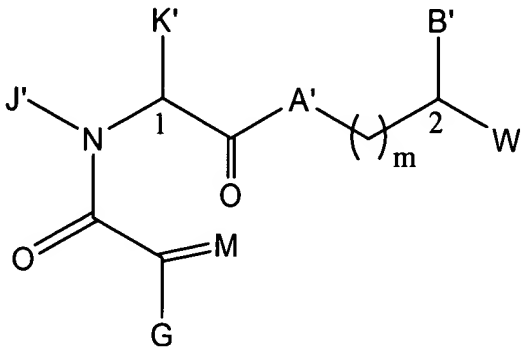
- wherein n is 0-3.

OR

Claim 6 of U.S. Patent No. 6,037,370

A method for stimulating neurite growth in nerve cells comprising the step of contacting said nerve cells with a composition comprising a neurotrophic amount of a compound having the formula (II):

(II)



and pharmaceutically acceptable derivatives thereof, wherein A', B', G, W, Ar' and its optional substituents, M, X, Y and its optional substituents, R, T', Q', U', q and m are as defined in claim 1;

{Beginning of claim 1 definitions.}

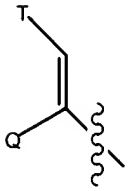
A' is CH_2, oxygen, NH or N-(C1-C4 alkyl);

B' and W are independently:

(i) hydrogen, Ar', (C1-C10)-straight or branched alkyl, (C2-C10)-straight or branched alkenyl or alkynyl, (C5-C7)-cycloalkyl-substituted (C1-C6)-straight or branched alkyl, (C5-C7)-cycloalkyl-substituted (C2-C6)-straight or branched alkenyl or alkynyl, (C5-C7)-cycloalkenyl-substituted (C1-C6)-straight or branched alkyl, (C5-C7)-cycloalkenyl-substituted (C2-C6) straight or branched alkenyl or alkynyl, Ar'-substituted-(C1-C6)-straight or branched alkyl, or Ar'-substituted-(C2-C6)-straight or branched alkenyl or alkynyl; wherein, in each case, any one of the CH_2 groups of said alkyl, alkenyl, or alkynyl chains may be optionally replaced by a heteroatom selected from the group consisting of O, S, SO, SO_2, N, and NR,

wherein R is selected from the group consisting of hydrogen; (C1-C4)-straight or branched alkyl; (C2-C4)-straight or branched alkenyl or alkynyl; (C1-C4) bridging alkyl, wherein a bridge is formed between the nitrogen and a carbon atom of said heteroatom-containing chain to form a ring, and wherein said ring is optionally fused to an Ar' group; or

(ii)



wherein Q' is hydrogen, (C1-C6)-straight or branched alkyl or (C2-C6)-straight or branched alkenyl or alkynyl; and

T' is Ar' or a 5-7 membered cycloalkyl ring with substituents at positions 3 and 4, said substituents being independently selected from oxo, hydrogen, hydroxyl, O-(C1-C4)-alkyl, or O-(C2-C4)-alkenyl;

wherein Ar' is selected from phenyl, 1-naphthyl, 2-naphthyl, indenyl, azulenyl, fluorenyl, anthracenyl, 2-furyl, 3-furyl, 2-thienyl, 3-thienyl, 2-pyridyl, 3-pyridyl, 4-pyridyl, pyrrolyl, oxazolyl, thiazolyl, imidazolyl, pyrazolyl, isoxazolyl, isothiazolyl, 1,2,3-oxadiazolyl, 1,2,3-tiazolyl, 1,3,4-thiadiazolyl, pyridazinyl, pyrimidinyl, pyrazinyl, 1,3,5-triazinyl, 1,3,5-trithianyl, indolinyl, isoindolyl, 3H-indolyl, indolinyl, bebzo[b]furanyl, benzo[b]thiophenyl, 1H-indazolyl, benzimidazolyl, benzthiazolyl, purinyl, 4H-quinolizinyl, quinolinyl, isoquinolinyl, cinnolinyl, phthalazinyl, quinazolinyl, quinoxalinyl, 1,8-naphthyridinyl, pteridinyl, carbazolyl, acridnyl, phenazinyl, phenothiazinyl, phenoxazinyl; and

wherein Ar' optionally contains 1-3 substituents which are independently selected from: halogen, hydroxyl, hydroxymethyl, nitro, trifluoromethyl, trifluoromethoxy, C1-C6 straight or branched alkyl, C2-C6 straight or branched alkenyl, O-(C1-C4) straight or branched alkyl, O-

(C2-C4) straight or branched alkenyl, O-benzyl, O-phenyl, amino, 1,2-methylenedioxy, carboxyl, N-[(C1-C5) straight or branched alkyl or (C2-C5) straight or branched alkenyl]-carboxamide, N-morpholinocarboxamide, N-benzylcarboxamide, N-thiomorpholinocarboxamide, N-picolinoylcarboxamide, O–X, CH_2–$(CH_2)_q$–X, O–$(CH_2)_q$–X, $(CH_2)_q$–O–X, CH=CH–X

wherein X is 4-methoxyphenyl, 2-pyridyl, 3-pyridyl, 4-pyridyl, pyrazyl, quinolyl, 3,5-dimethylisoxazoyl, isoxazoyl, 2-methylthiazoyl, thiazoyl, 2-thienyl, 3-thienyl, pyrimidyl; and

q is 0-2;

G is U';

M is either oxygen or CH-U; provided that if G is hydrogen, then M is CH-U' or if M is oxygen, then U' is not hydrogen;

wherein U' is hydrogen, O-[(C1-C4) straight or branched alkyl], O-[(C2-C4) straight or branched alkenyl], (C1-C6) straight or branched alkyl, (C2-C6) straight or branched alkenyl, (C5-C7) cycloalkyl or (C5-C7)-cycloalkenyl substituted with C1-C4 straight or branched alkyl or C2-C4 straight or branched alkenyl, [(C1-C4 alkyl or (C2-C4) alkenyl]-Y or Y;

wherein Y is selected from the group consisting of phenyl, 1-naphthyl, 2-naphthyl, indenyl, azulenyl, fluorenyl, anthracenyl, 2-pyrrolinyl, 3-pyrrolinyl, pyrrolidinyl, 1,3-dioxolyl, 2-imidazolinyl, imidazolidinyl, 2H-pyranyl, 4H-pyranyl, piperidyl,

1,4-dioxanyl, morpholinyl, 1,4-dithianyl, thiomorpholinyl, piperazinyl, quinuclidinyl, 2-furyl, 3-furyl, 2-thienyl, 3-thienyl, 2-pyridyl, 3-pyridyl, 4-pyridyl, pyrrolyl, oxazolyl, thiazolyl, imidazolyl, pyrazolyl, isoxazolyl, isothiazolyl, 1,2,3-oxadiazolyl, 1,2,3-tiazolyl, 1,3,4-thiadiazolyl, pyridazinyl, pyrimidinyl, pyrazinyl, 1,3,5-triazinyl, 1,3,5-trithianyl, indolizinyl, indolyl, isoindolyl, 3H-indolyl, indolinyl, bebzo[b]furanyl, benzo[b]thiophenyl, 1H-indazolyl, benzimidazolyl, benzthiazolyl, purinyl, 4H-quinolizinyl, quinolinyl, isoquinolinyl, cinnolinyl, phthalazinyl, quinazolinyl, quinoxalinyl, 1,8-naphthyridinyl, pteridinyl, carbazolyl, acridnyl, phenazinyl, phenothiazinyl, phenoxazinyl

wherein Y optionally contains 1-3 substituents which are independently selected from halogen, hydroxyl, hydroxymethyl, nitro, trifluoromethyl, trifluoromethoxy, C1-C6 straight or branched alkyl, C2-C6 straight or branched alkenyl, O-(C1-C4) straight or branched alkyl, O-(C2-C4) straight or branched alkenyl, O-benzyl, O-phenyl, 1,2-methylenedioxy, amino, or carboxyl;

m is 0-3

{End of claim 1 definitions.}

and

J' and K' are taken together with the nitrogen atom and the carbon atom to which they are respectively bound to form a 5-membered heterocyclic ring;

provided that if G is hydrogen, then M is CH-U' or if M is oxygen, then G is not hydrogen; and

provided that when:

M is oxygen;

A is oxygen, NH or N-(C1-C4 alkyl);

G is (C1-C6) straight or branched alkyl, (C2-C6) straight or branched alkenyl, (C5-C7) cycloalkyl or (C5-C7) cycloalkenyl substituted with C1-C4 straight or branched alkyl or C2-C4 straight or branched alkenyl, or Y; and

Y is 1-napthyl, 2-napthyl, indolyl, 2-furyl, 3-furyl, thiazolyl, 2-thienyl, 3, thienyl, 2- pyridyl, 3-pyridyl, 4-pyridyl or phenyl;

then $(CH_2)_m$ B' and W, taken together, do not form:

i) substituted or unsubstituted indolyl, 2-furyl, 3-furyl, thiazolyl, 2-thienyl, 3-thienyl, 2-pyridyl, 3-pyridyl, 4-pyridyl; phenyl

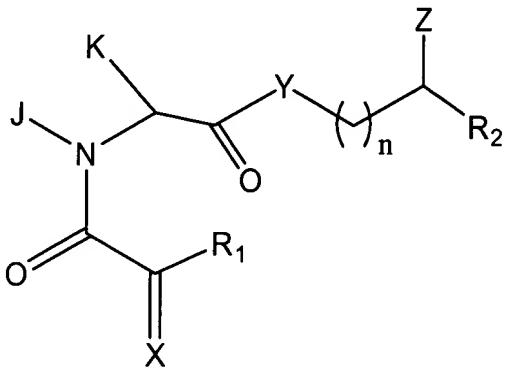
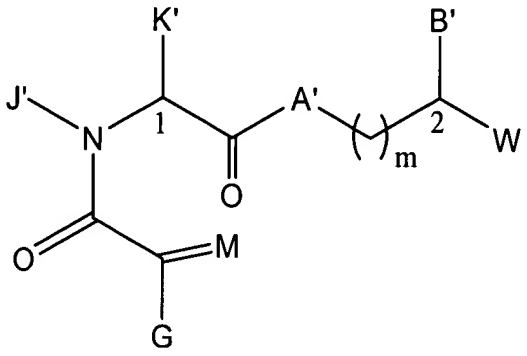
ii) an alkyl or alkenyl chain substituted with substituted or unsubstituted indolyl, 2-furyl, 3-furyl, thiazolyl, 2-thienyl, 3-thienyl, 2-pyridyl, 3-pyridyl, 4-pyridyl or phenyl or

iii) an alkyl or alkenyl chain substituted with (C5-C7) cycloalkyl.

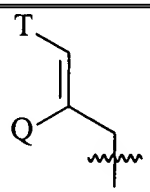
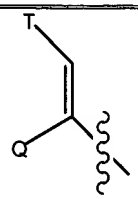
EXHIBIT C

COMPARISON OF THE CLAIMS FORMING THE COUNT:

Claim 41 of Serial No. 09/805,249 and Claim 6 of US 6,037,370

Claim 41 (09/805,249)	**Claim 6 (US 6,037,370)**
A method for stimulating neurite outgrowth by a nerve cell comprising:	A method for stimulating neurite growth in nerve cells comprising the step of
administering to said nerve cell an effective amount of compound having an affinity for FKBP-type immunophilins according to formula I	contacting said nerve cells with a composition comprising a neurotrophic amount of a compound having the formula (II):
	
or a pharmaceutically acceptable salt thereof, wherein	and pharmaceutically acceptable derivatives thereof, wherein A', B', G, W, Ar' and its optional substituents, M, X, Y and its optional substituents, R, T', Q', U', q and m are as defined in claim 1; *{Claim 1 definitions appear below in italics.}*
Y is **CH_2, oxygen, NH, N-(C1-C4 alkyl);**	***A' is CH_2, oxygen, NH, N-(C1-C4 alkyl);***
wherein Z and R_2 are independently **Ar**	*B' and W are independently* *H* ***Ar'*** *C1-C10 straight or branched alkyl* *C2-C10 straight or branched alkenyl* *C2-C10 straight or branched alkynyl*

Claim 41 (09/805,249)	Claim 6 (US 6,037,370)
(C5-C7)-cycloalkyl substituted (C1-C6)-straight or branched alkyl or alkenyl **(C5-C7)-cycloalkenyl substituted (C1-C6)-straight or branched alkyl or alkenyl** **Ar substituted (C1-C6)-straight or branched alkyl or alkenyl** wherein in each case, one or two carbon atoms of the straight or branched alkyl or alkenyl groups may be substituted with 1-2 heteroatoms selected from the group consisting of **oxygen, sulfur, SO and SO_2** in chemically reasonable substitution patterns,	***(C5-C7) cycloalkyl-substituted C1-C6 straight or branched alkyl*** ***(C5-C7) cycloalkyl-substituted C2-C6 straight or branched alkenyl*** *(C5-C7) cycloalkyl-substituted C2-C6 straight or branched alkynyl* ***(C5-C7) cycloalkenyl-substituted C1-C6 straight or branched alkyl*** ***(C5-C7) cycloalkenyl-substituted C2-C6 straight or branched alkenyl*** *(C5-C7) cycloalkenyl-substituted C1-C6 straight or branched alkynyl* ***Ar'-substituted C1-C6 straight or branched alkyl*** ***Ar'-substituted C2-C6 straight or branched alkenyl*** *Ar'-substituted C2-C6 straight or branched alkynyl* *wherein, in each case, any one of the CH_2 groups of said alkyl, alkenyl or alkynyl chains may be optionally replaced by a heteratom selected from the group consisting of **O, S, SO, SO_2**, N and NR,*
	wherein R is selected from the group consisting of *hydrogen* *(C1-C4) straight or branched alkyl* *(C2-C4) straight or branched alkenyl* *(C2-C4) straight or branched alkynyl* *(C1-C4) bridging alkyl wherein a bridge is formed between the nitrogen and a carbon atom of said heteroatom-containing chain to form a ring, and wherein said ring is optionally fused to an Ar' group;* *or*

Claim 41 (09/805,249)	Claim 6 (US 6,037,370)
 or	
wherein Q is **hydrogen,** **(C1-C6)-straight or branched alkyl** **or** **(C1-C6)-straight or branched alkenyl;**	***wherein Q' is*** ***hydrogen*** ***(C1-C6) straight or branched alkyl*** ***(C2-C6) straight or branched alkenyl*** *(C2-C6) straight or branched alkynyl;*
wherein T is **Ar or** **substituted 5-7 membered cycloalkyl with substituents at positions 3 and 4 which are independently selected from the group consisting of** **hydrogen** **hydroxyl** **O-(C1-C4)-alkyl or alkenyl** carbonyl;	*T is* ***Ar'*** ***5-7 membered cycloalkyl ring with substituents at positions 3 and 4, said substituents being independently selected from*** *oxo* ***hydrogen*** ***hydroxyl*** ***O-(C1-C4) alkyl*** ***O-(C2-C4) alkenyl;***
wherein Ar is selected from the group consisting of **monocyclic and bicyclic heterocyclic aromatic ring systems with individual ring sizes being 5 or 6** **which may contain in either or both rings a total of 1-4 heteroatoms independently selected from oxygen nitrogen sulfur;**	*wherein Ar' is selected from* ***phenyl*** *1-naphthyl* *2-naphthyl* *indenyl* *azulenyl* *fluorenyl* *anthracenyl* *2-furyl* *3-furyl* *2-thienyl* *3-thienyl* *2-pyridyl* *3-pyridyl* *4-pyridyl* *pyrrolyl* *oxazolyl* *thiazolyl*

Claim 41 (09/805,249)	Claim 6 (US 6,037,370)
	imidazolyl *pyrazolyl* *isoxazolyl* *isothiazolyl* *1,2,3-oxadiazolyl* *1,2,3-triazolyl* *1,3,4-thiadiazolyl* *pyridazinyl* *pyrimidinyl* *pyrazinyl* *1,3,5-triazinyl* *1,3,5-trithianyl* ***indolizinyl*** ***indolyl*** ***isoindolyl*** ***3H-indolyl*** ***indolinyl*** ***benzo[b]furanyl*** ***benzo[b]thiophenyl*** ***1H-indazolyl*** ***benzimidazolyl*** ***benzthiazolyl*** ***purinyl*** ***4H-quinolizinyl*** ***quinolinyl*** ***isoquinolinyl*** ***cinnolinyl*** ***phthalazinyl*** ***quinazolinyl*** ***quinoxalinyl*** ***1,8-naphthyridinyl*** ***pteridinyl*** *carbazolyl* *acridinyl* *phenazinyl* *phenothiazinyl* *phenoxazinyl;*
wherein Ar may contain one to three substituents which are independently selected from the group consisting of **hydrogen** **halo**	*wherein Ar' optionally contains 1-3 substituents which are independently selected from:* ***halogen***

Claim 41 (09/805,249)	Claim 6 (US 6,037,370)
hydroxyl **hydroxymethyl** **nitro** **CF_3** **trifluoromethoxy** **C1-C6 straight or branched alkyl** **C1-C6 straight or branched alkenyl** **O-(C1-C4) straight or branched alkyl** **O-(C1-C4) straight or branched alkenyl** **O-benzyl** **O-phenyl** **amino** **1,2-methylenedioxy** carbonyl phenyl;	***hydroxyl*** ***hydroxymethyl*** ***nitro*** ***trifluoromethyl*** ***trifluoromethoxy*** ***C1-C6 straight or branched alkyl*** ***C2-C6 straight or branched alkenyl*** ***O-(C1-C4) straight or branched alkyl*** ***O-(C2-C4) straight or branched alkenyl*** ***O-benzyl*** ***O-phenyl*** ***1,2-methylenedioxy*** ***amino*** *carboxyl* *N-[C1-C5 straight or branched alkyl]-carboxamide* *N-[C2-C5 straight or branched alkenyl]-carboxamide* *N, N-di-[C1-C5 straight or branched alkyl]-carboxamide* *N, N-di-[C2-C5 straight or branched alkenyl]-carboxamide* *N-morpholinocarboxamide* *N-benzylcarboxamide* *N-thiomorpholinocarboxamide* *N-picolinoylcarboxamide* *O–X* *CH_2–$(CH_2)_q$–X* *O–$(CH_2)_q$–X* *$(CH_2)_q$–O–X* *CH=CH–X* *wherein X is* *4-methoxyphenyl* *2-pyridyl* *3-pyridyl* *4-pyridyl* *pyrazyl* *quinolyl* *3,5-dimethylisoxazoyl* *isoxazoyl* *2-methylthiazoyl*

Claim 41 (09/805,249)	Claim 6 (US 6,037,370)
	thiazoyl *2-thienyl* *3-thienyl* *pyrimidyl;* *and q is 0-2*
wherein R_1 is either hydrogen or **U;**	*G is **U';***
X is either **oxygen or CH-U;**	*M is either **oxygen or CH-U';***
(Provisio moved from here to aid comparison)	
wherein U is **hydrogen** **O-(C1-C4)-straight or branched alkyl** **O-(C1-C4)-straight or branched alkenyl** **(C1-C6)-straight or branched alkyl** **(C1-C6)-straight or branched alkenyl** (C5-C7)-cycloalkyl **(C5-C7)-cycloalkenyl substituted with** **(C1-C4)-straight or branched alkyl** **(C1-C4)-straight or branched alkenyl** **[(C1-C4)-alkyl or alkenyl]-Ar** **Ar (Ar as described above);**	*wherein U' is* ***H*** ***O-[(C1-C4) straight or branched alkyl]*** ***O-[(C2-C4) straight or branched alkenyl]*** ***C1-C6 straight or branched alkyl*** ***C2-C6 straight or branched alkenyl*** *C5-C7 cycloalkyl substituted with:* *C1-C4 straight or branched alkyl* *C2-C4 straight or branched alkenyl* ***C5-C7 cycloalkenyl substituted with:*** ***C1-C4 straight or branched alkyl*** ***C2-C4 straight or branched alkenyl*** ***[(C1-C4 alkyl) or (C2-C4 alkenyl)]-Y*** ***Y;***
{Ar definition is repeated below in italics.} *wherein Ar is selected from the group consisting of* *monocyclic and bicyclic heterocyclic aromatic ring systems with individual ring sizes being 5 or 6*	*wherein Y is selected from the group consisting of* *phenyl* *1-naphthyl* *2-naphthyl*

Claim 41 (09/805,249)	Claim 6 (US 6,037,370)
which may contain in either or both rings a total of 1-4 heteroatoms independently selected from oxygen, nitrogen, sulfur	*indenyl* *azulenyl* *fluorenyl* *anthracenyl* *2-pyrrolinyl* *3-pyrrolinyl* *pyrrolidinyl* *1,3-dioxolyl* *2-imidazolinyl* *imidazolidinyl* *2H-pyranyl* *4H-pyranyl* *piperidyl* *1,4-dioxanyl* *morpholinyl* *1,4-dithianyl* *thiomorpholinyl* *piperazinyl* *quinuclidinyl* *2-furyl* *3-furyl* *2-thienyl* *3-thienyl* *2-pyridyl* *3-pyridyl* *4-pyridyl* *pyrrolyl* *oxazolyl* *thiazolyl* *imidazolyl* *pyrazolyl* *isoxazolyl* *isothiazolyl* *1,2,3-oxadiazolyl* *1,2,3-tiazolyl* *1,3,4-thiadiazolyl* *pyridazinyl* *pyrimidinyl* *pyrazinyl* *1,3,5-triazinyl* *1,3,5-trithianyl* *indolizinyl*

Claim 41 (09/805,249)	Claim 6 (US 6,037,370)
	indolyl *isoindolyl* *3H-indolyl* *indolinyl* *bebzo[b]furanyl* *benzo[b]thiophenyl* *1H-indazolyl* *benzimidazolyl* *benzthiazolyl* *purinyl* *4H-quinolizinyl* *quinolinyl* *isoquinolinyl* *cinnolinyl* *phthalazinyl* *quinazolinyl* *quinoxalinyl* *1,8-naphthyridinyl* *pteridinyl* *carbazolyl* *acridnyl* *phenazinyl* *phenothiazinyl* *phenoxazinyl;*
wherein Ar may contain one to three substituents which are independently selected from the group consisting of	***wherein Y optionally contains 1-3 substituents which are independently selected from:***
hydrogen ***halo*** ***hydroxyl*** ***hydroxymethyl*** ***nitro*** ***CF_3*** ***trifluoromethoxy*** ***C1-C6 straight or branched alkyl*** ***C2-C6 straight or branched alkenyl*** ***O-(C1-C4) straight or branched alkyl*** ***O-(C2-C4) straight or branched alkenyl*** ***O-benzyl***	***halogen*** ***hydroxyl*** ***hydroxymethyl*** ***nitro*** ***trifluoromethyl*** ***trifluoromethoxy*** ***C1-C6 straight or branched alkyl*** ***C2-C6 straight or branched alkenyl*** ***O-(C1-C4) straight or branched alkyl*** ***O-(C2-C4) straight or branched alkenyl*** ***O-benzyl***

Claim 41 (09/805,249)	Claim 6 (US 6,037,370)
O-phenyl ***amino*** ***1,2-methylenedioxy*** *carbonyl* *phenyl* *{End of repeated Ar definition.}*	***O-phenyl*** ***1,2-methylenedioxy*** ***amino*** *carboxyl;* *{End of claim 1 definitions.}*
wherein J is hydrogen, C1 alkyl, C2 alkyl, benzyl; K is (C1-C4)-straight or branched alkyl, benzyl, cyclohexylethyl; or	
wherein **J and K may be taken together to form a 5 membered heterocyclic ring** which may contain an oxygen (O) sulfur (S) SO SO_2 substituted therein;	**J' and K' are taken together with the nitrogen atom and the carbon atom to which they are respectively bound to form a 5-membered heterocyclic ring;**
(Provisio moved from above to aid comparison) provided that if **R_1 is hydrogen then** **X is CH-U** or if X is oxygen then R_1 is U;	 provided that if **G is hydrogen, then** **M is CH-U'** or if M is oxygen, then G is not hydrogen; and
	provided[1] that when: M is O A' is O, NH or N-(C1-C4 alkyl) G is (C1-C6) straight or branched alkyl, (C2-C6) straight or branched alkenyl, (C5-C7) cycloalkyl or (C5-C7) cycloalkenyl substituted with C1-C4 straight or branched alkyl or C2-

[1] This proviso excludes a relatively small sub-genus.

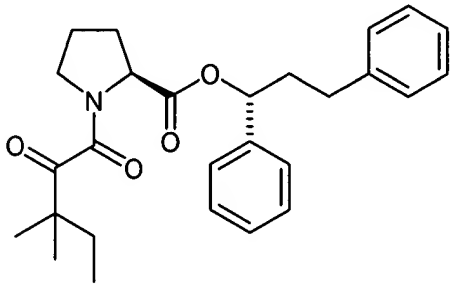
Claim 41 **(09/805,249)**	**Claim 6** **(US 6,037,370)**
	C4 straight or branched alkenyl, or Y; and Y is 1-napthyl, 2-napthyl, indolyl, 2-furyl, 3-furyl, thiazolyl, 2-thienyl, 3, thienyl, 2-pyridyl, 3-pyridyl, 4-pyridyl or phenyl; then $(CH_2)_m$ B' and W, taken together, do not form: i) substituted or unsubstituted indolyl, 2-furyl, 3-furyl, thiazolyl, 2-thienyl, 3-thienyl, 2-pyridyl, 3-pyridyl, 4-pyridyl; phenyl ii) an alkyl or alkenyl chain substituted with substituted or unsubstituted indolyl, 2-furyl, 3-furyl, thiazolyl, 2-thienyl, 3-thienyl, 2-pyridyl, 3-pyridyl, 4-pyridyl or phenyl or iii) an alkyl or alkenyl chain substituted with (C5-C7) cycloalkyl.
wherein **n is 0-3.**	***m is 0-3 (as defined in claim 1).***

EXHIBIT D

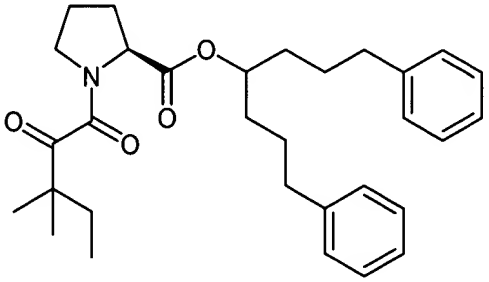
CLAIM CHART: CONSTRUCTIVE REDUCTION TO PRACTICE OF TWO (2) SPECIES

At least two (2) compounds benefiting from the June 7, 1995, filing date of application 08/479,436 (the earliest priority application) fall within the scope of the count and serve as constructive reduction to practice of the count.

Species 1 and 2 below are disclosed in the present application and in 09/359,351; 08/693,003; and 08/479,436 (the priority applications)[1]:

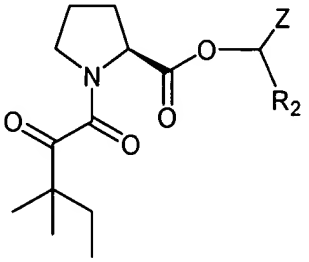


Species 1[2]



Species 2[3]

Species 1 and 2 share the core structure shown below:



Core Structure

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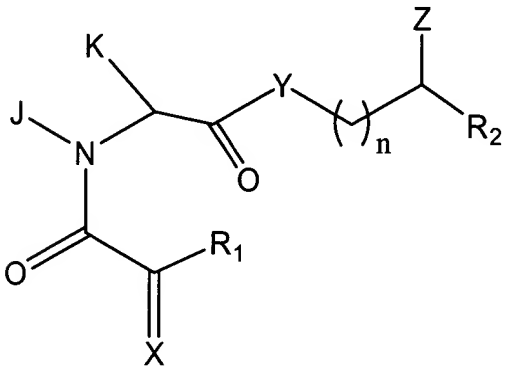
[1] The priority applications have identical disclosures.

[2] See the present application, Example 11, p. 29; priority applications, Example 10, p. 38, l. 13.

[3] 1,7-diphenyl-4-heptyl (2*S*)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate. See the present application, Example 17, p. 32; priority applications, p. 19, ll. 19-20, and Example 4, p. 34.

This core structure corresponds to the compounds of Formula I of claim 41, which is recited in the alternative in the count, wherein Z and R_2 vary and the other variables are as shown in the table below.



Formula I

- X and Y are each oxygen
- R_1 is U and U is 1,1-dimethylpropane
- J and K form a pyrrolidine ring
- n is zero

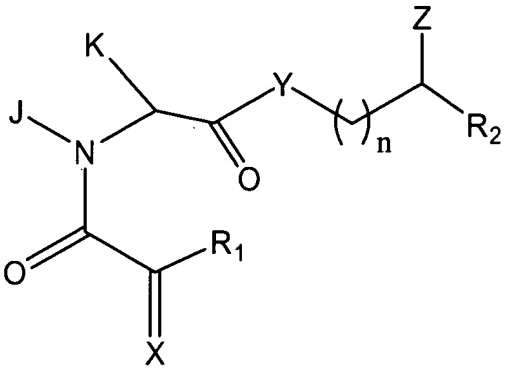
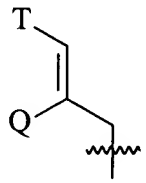
Species 1: Z is phenyl and R_2 is phenylethyl or "Ar'-substituted (C1-C6) straight or branched alkyl or alkenyl."

Species 2: Z and R_2 are both phenylpropyl or "Ar'-substituted (C1-C6) straight or branched alkyl or alkenyl."

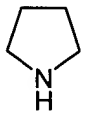
The following claim chart shows how Species 1 and 2 fall within the scope of the count, i.e., within the scope of claim 41 of the present application. The proposed count is "claim 41 of the present application or claim 6 of U.S. Patent No. 6,037,370."

Claim 41 (09/805,249)	**Disclosures In 08/479,436[4] (US 5,614,547)**
A method for stimulating neurite outgrowth by a nerve cell comprising:	"The neurotrophic compounds of this invention can be periodically administered to a patient undergoing treatment for neurological disorders or for other reasons in which it is desirable to stimulate neuronal regeneration and growth . . ." (p. 21, ll. 10-14; present application, p. 13, ll. 11-13)
administering to said nerve cell an effective amount of compound having an affinity for FKBP-type immunophilins according to formula I	"This invention relates to neurotrophic compounds having an affinity for FKBP-type immunophilins . . ." (p. 1, ll. 3-4; present application, p. 1, ll. 7-8)

[4] Citations are identical for the priority applications 09/359,351; 08/693,003; and 08/479,436, which have identical disclosures.

Claim 41 (09/805,249)	Disclosures In 08/479,436[4] (US 5,614,547)
	Species 1 and 2
or a pharmaceutically acceptable salt thereof, wherein	
Y is CH_2 oxygen NH N-(C1-C4 alkyl)	Y is oxygen
wherein **Z** and **R_2** are independently Ar (C5-C7)-cycloalkyl substituted (C1-C6)-straight or branched alkyl or alkenyl (C5-C7)-cycloalkenyl substituted (C1-C6)-straight or branched alkyl or alkenyl Ar substituted (C1-C6)-straight or branched alkyl or alkenyl wherein in each case, one or two carbon atoms of the straight or branched alkyl or alkenyl groups may be substituted with 1-2 heteroatoms selected from the group consisting of oxygen, sulfur, SO and SO_2 in chemically reasonable substitution patterns, or	<u>Species 1</u>: Z = phenyl R_2 = Ar substituted (C1-C6)-straight alkyl <u>Species 2</u>: Z, R_2 = Ar substituted (C1-C6)-straight alkyl
	
- wherein Q is hydrogen, (C1-C6)-	

Claim 41 (09/805,249)	Disclosures In 08/479,436[4] (US 5,614,547)
straight or branched alkyl or (C1-C6)-straight or branched alkenyl	
- wherein T is Ar or substituted 5-7 membered cycloalkyl with substituents at positions 3 and 4 which are independently selected from the group consisting of hydrogen, hydroxyl, O-(C1-C4)-alkyl or O-(C1-C4)-alkenyl and carbonyl	
- wherein Ar is selected from the group consisting of monocyclic and bicyclic heterocyclic aromatic ring systems with individual ring sizes being 5 or 6, which may contain in either or both rings a total of 1-4 heteroatoms independently selected from oxygen, nitrogen and sulfur; wherein Ar may contain one to three substituents which are independently selected from the group consisting of hydrogen, halo, hydroxyl, hydroxymethyl, nitro, CF_3, trifluoromethoxy, (C1-C6)-straight or branched alkyl or (C1-C6)-straight or branched alkenyl, O-(C1-C4)-straight or branched alkyl or O-(C1-C4)-straight or branched alkenyl, O-benzyl, O-phenyl, amino, 1,2-methylenedioxy, carbonyl and phenyl;	
wherein $\mathbf{R_1}$ is either hydrogen or U	R_1 is U
X is either oxygen or CH-U	X is oxygen
provided that if R_1 is hydrogen then X is CH-U or if X is oxygen then R_1 is U	
wherein **U** is hydrogen O-(C1-C4)-straight or branched alkyl O-(C1-C4)-straight or branched alkenyl (C1-C6)-straight or branched alkyl	 U is 1,1-dimethylpropyl

Claim 41 (09/805,249)	Disclosures In 08/479,436[4] (US 5,614,547)
(C1-C6)-straight or branched alkenyl (C5-C7)-cycloalkyl (C5-C7)-cycloalkenyl substituted with (C1-C4)-straight or branched alkyl (C1-C4)-straight or branched alkenyl [(C1-C4)-alkyl or alkenyl]-Ar Ar (Ar as described above)	
- wherein J is hydrogen or C1 or C2 alkyl or benzyl K is (C1-C4)-straight or branched alkyl, benzyl or cyclohexylethyl; or	
wherein **J and K** may be taken together to form a 5 membered heterocyclic ring which may contain an oxygen (O), sulfur (S), SO or SO_2 substituted therein; and	J and K together form a pyrrolidine ring: 
n is 0-3	n = 0

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(rev. 6-99)

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APPLICATION NUMBER	FILING DATE	GRP ART UNIT	FIL FEE REC'D	ATTORNEY DOCKET NO.	DRWGS	TOT CL	IND CL
09/359,351	07/21/99	1613	$653.00	22762-YY	0	33	7

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WASHINGTON DC 20005

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Applicant(s) GREGORY S. HAMILTON, CATONSVILLE, MD; JOSEPH P. STEINER, HAMPSTEAD, MD.

CONTINUING DATA AS CLAIMED BY APPLICANT-
THIS APPLN IS A CON OF 08/693,003 08/06/96

IF REQUIRED, FOREIGN FILING LICENSE GRANTED 08/10/99 ** SMALL ENTITY **
TITLE
SMALL MOLECULE INHIBITORS OF ROTAMASE ENZYME ACTIVITY

PRELIMINARY CLASS: 514

DATA ENTRY BY: BURNS, ERIC TEAM: 07 DATE: 08/10/99

(See reverse for new important information)

Attorney Docket 22762-YY

Title:

SMALL MOLECULE INHIBITORS OF ROTAMASE ENZYME ACTIVITY

Inventor: Gregory S. Hamilton
Joseph Steiner

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BACKGROUND OF THE INVENTION

1. Field of the Invention

This invention relates to neurotrophic compounds having an affinity for FKBP-type immunophilins, their preparation and use as inhibitors of the enzyme activity associated with immunophilin proteins, and particularly inhibitors of peptidyl-prolyl isomerase or rotamase enzyme activity.

2. Description of the Prior Art

The term immunophilin refers to a number of proteins that serve as receptors for the principal immunosuppressant drugs, cyclosporin A (CsA), FK506, and rapamycin. Known classes of immunophilins are cyclophilins, and FK506 binding proteins, such as FKBP. Cyclosporin A binds to cyclophilin while FK506 and rapamycin bind to FKBP. These immunophilin-drug complexes interface with a variety of intracellular signal transduction systems, especially in the immune system and the nervous system.

Immunophilins are known to have peptidyl-prolyl isomerase (PPIase) or rotamase enzyme activity. It has been determined that rotamase activity has a role in the catalyzation of the interconversion of the cis and trans isomer of immunophilin proteins.

Immunophilins were originally discovered and studied in immune tissue. It was initially postulated by those skilled in the art that inhibition of the immunophilins rotamase activity leads to the inhibition

of T-cell proliferation, thereby causing the immunosuppressive action exhibited by immunosuppressive drugs such as cyclosporin A, FK506, and rapamycin. Further study has shown that the inhibition of rotamase activity, in and of itself, is not sufficient for immunosuppressant activity. Schreiber et al., *Science*, 1990 vol. 250 pp. 556-559. It has been shown that the immunophilin-drug complexes interact with ternary protein targets as their mode of action. Schreiber et al., *Cell*, 1991, vol. 66, pp. 807-815. In the case of FKBP-FK506 and FKBP-CsA, the drug-immunophilin complexes bind to the enzyme calcineurin, inhibitory T-cell receptor signalling leading to T-cell proliferation. Similarly, the complex of rapamycin and FKBP interacts with the RAFT1/FRAP protein and inhibits signalling from the IL-2 receptor.

Immunophilins have been found to be present at high concentrations in the central nervous system. Immunophilins are enriched 10-50 times more in the central nervous system than in the immune system. Within neural tissues, immunophilins appear to influence neuronal process extension, nitric oxide synthesis, and neurotransmitter release.

It has been found that picomolar concentrations of an immunosuppressant such as FK506 and rapamycin stimulate neurite out growth in PC12 cells and sensory nervous, namely dorsal root ganglion cells (DRGs). Lyons et al., *Proc. of Natl. Acad. Sci.*, 1994 vol. 91,

pp. 3191-3195. In whole animal experiments, FK506 has been shown to stimulate nerve regeneration following facial nerve injury and results in functional recovery in animals with sciatic nerve lesions.

Surprisingly, it has been found that drugs with a high affinity for FKBP are potent rotamase inhibitors causing a neurotrophic effect... Lyons et al. These findings suggest the use of immunosuppressants in treating various peripheral neuropathies and enhancing neuronal regrowth in the central nervous system (CNS). Studies have demonstrated that neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis (ALS) may occur due to the loss, or decreased availability, of a neurotrophic substance specific for a particular population of neurons affected in the disorder.

Several neurotrophic factors effecting specific neuronal populations in the central nervous system have been identified. For example, it has been hypothesized that Alzheimer's disease results from a decrease or loss of nerve growth factor (NGF). It has thus been proposed to treat Alzheimer's patients with exogenous nerve growth factor or other neurotrophic proteins such as brain derived nerve factor (BDNF), glial derived nerve factor, ciliary neurotrophic factor, and neurotropin-3 to increase the survival of degenerating neuronal populations.

Clinical application of these proteins in various neurological disease states is hampered by difficulties in the delivery and bioavailability of large proteins to nervous system targets. By contrast, immunosuppressant drugs with neurotrophic activity are relatively small and display excellent bioavailability and specificity. However, when administered chronically, immunosuppressants exhibit a number of potentially serious side effects including nephrotoxicity, such as impairment of glomerular filtration and irreversible interstitial fibrosis (Kopp et al., 1991, J. Am. Soc. Nephrol. 1:162); neurological deficits, such as involuntary tremors, or non-specific cerebral angina such as non-localized headaches (De Groen et al., 1987, N. Engl. J. Med. 317:861); and vascular hypertension with complications resulting therefrom (Kahan et al., 1989 N. Engl. J. Med. 321: 1725).

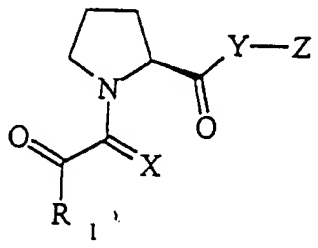
In order to prevent the side effects associated with use of the immunosuppressant compounds, the present invention provides non-immunosuppressive compounds containing small molecule FKBP rotamase inhibitors for promoting neuronal growth and regeneration in various neuropathological situations where neuronal repair can be facilitated including peripheral nerve damage by physical injury or disease state such as diabetes, physical damage to the central nervous system (spinal cord and brain) brain damage

associated with stroke, and for the treatment of neurological disorders relating to neurodegeneration, including Parkinson's disease, Alzheimer's disease, and amyotrophic lateral sclerosis.

SUMMARY OF THE INVENTION

The present invention relates to a novel class of neurotrophic compounds having an affinity for FKBP-type immunophilins. Once bound to this protein the neurotrophic compounds are potent inhibitors of the enzyme activity associated with immunophilin proteins and particularly rotamase enzyme activity, thereby stimulating neuronal regeneration and outgrowth. A key feature of the compounds of the present invention is that they do not exert any significant immunosuppressive activity in addition to their neurotrophic activity.

A preferred embodiment of this invention is a neurotrophic compound of the formula:



where

R_1 is selected from the group consisting of a C_1-C_9 straight or branched chain alkyl or alkenyl group optionally substituted with C_3-C_8 cycloalkyl, C_3 or C_5 cycloalkyl, C_5-C_7 cycloalkenyl, Ar_1, where said alkyl, alkenyl, cycloalkyl or cycloalkenyl groups may be optionally substituted with C_1-C_4 alkyl, C_1-C_4 alkenyl, or hydroxy, where Ar_1 is selected from the group consisting of 1-napthyl, 2-napthyl, 2-indolyl, 3-indolyl, 2-furyl, 3-furyl, 2-thiazolyl, 2-thienyl, 3-thienyl, 2-,3-, 4-pyridyl, and phenyl, having one to three substituents which are independently selected from the group consisting of hydrogen, halo, hydroxyl, nitro, trifluoromethyl, C_1-C_6 straight or branched alkyl or alkenyl, C_1-C_4 alkoxy or C_1-C_4 alkenyloxy, phenoxy, benzyloxy, and amino;

X is selected from the group consisting of oxygen, sulfur, methylene (CH_2), or H_2;

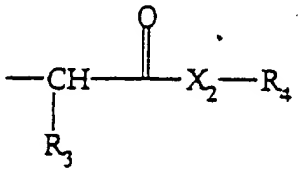
Y is selected from the group consisting of oxygen or NR_2, where R_2 is hydrogen or C_1-C_6 alkyl; and

Z is selected from the group consisting of C_2-C_6 straight or branched chain alkyl or alkenyl,

wherein the alkyl chain is substituted in one or more positions with Ar_1 as defined above, C_3-C_8 cycloalkyl, cycloalkyl connected by a C_1-C_6

straight or unbranched alkyl or alkenyl chain, and Ar_2 where Ar_2 is selected from the group consisting of 2-indolyl, 3-indolyl, 2-furyl, 3-furyl, 2- thiazolyl, 2-thienyl, 3-thienyl, 2-, 3-, or 4-pyridyl, and phenyl, having one to three substituents which are independently selected from the group consisting of hydrogen, halo, hydroxyl, nitro, trifluoromethyl, C_1-C_5 straight or branched alkyl or alkenyl, C_1-C_4 alkoxy or C_1-C_4 alkenyloxy, phenoxy, benzyloxy, and amino;

Z may also be the fragment:



where

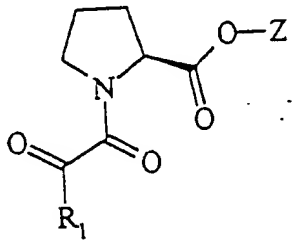
R_3 is selected from the group consisting of straight or branched alkyl C_1-C_8 optionally substituted with C_3-C_8 cycloalkyl, or Ar_1 as defined above, and unsubstituted Ar_1;

X_2 is O or NR_5, where R_5 is selected from the group consisting of hydrogen, C_1-C_6 straight or branched alkyl and alkenyl;

R_4 is selected from the group consisting of phenyl, benzyl, C_1-C_5 straight or branched alkyl or alkenyl, and C_1-C_5 straight or

branched alkyl or alkenyl substituted with phenyl; or pharmaceutically acceptable salts or hydrates thereof.

Another preferred embodiment of this invention is a neurotrophic compound of the formula:



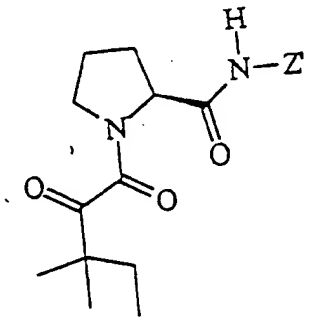
where

R_1 is a C_1-C_9 straight or branched chain alkyl or alkenyl group optionally substituted with C_3-C_8 cycloalkyl, C_3 or C_5 cycloalkyl, C_5-C_7 cycloalkenyl, or Ar_1, where said alkyl, alkenyl, cycloalkyl or cycloalkenyl groups may be optionally substituted with C_1-C_4 alkyl, C_1-C_4 alkenyl, or hydroxy, and where Ar_1 is selected from the group consisting of 1-napthyl, 2-napthyl, 2-indolyl, 3-indolyl, 2-furyl, 3-furyl, 2-thiazolyl, 2-thienyl, 3-thienyl, 2-,3-, or 4-pyridyl, or phenyl, having one to three substituents which are independently selected from the group consisting of hydrogen, halo, hydroxyl, nitro, trifluoromethyl, C_1-C_5 straight or branched alkyl or alkenyl, C_1-C_4 alkoxy or C_1-

C_4 alkenyloxy, phenoxy, benzyloxy, and amino;

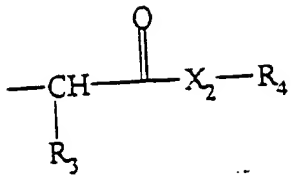
Z is a C_2-C_6 straight or branched chain alkyl or alkenyl, wherein the alkyl chain is substituted in one or more positions with Ar_1 as defined above, C_3-C_8 cycloalkyl, cycloalkyl connected by a C_1-C_6 straight or unbranched alkyl or alkenyl chain, or Ar_2 where Ar_2 is selected from the group consisting of 2-indolyl, 3-indolyl, 2-furyl, 3-furyl, 2- thiazolyl, 2-thienyl, 3-thienyl, 2-, 3-, or 4-pyridyl, or phenyl, having one to three substituents which are independently selected from the group consisting of hydrogen, halo, hydroxyl, nitro, trifluoromethyl, C_1-C_6 straight or branched alkyl or alkenyl, C_1-C_4 alkoxy or C_1-C_4 alkenyloxy, phenoxy, benzyloxy, and amino; or pharmaceutically acceptable salts or hydrates thereof.

Another preferred embodiment of this invention is a neurotrophic compound of the formula:



where

Z' is the fragment:



where

R_3 is selected from the group consisting of straight or branched alkyl C_1-C_8 optionally substituted with C_3-C_8 cycloalkyl, or Ar_1 as defined above, and unsubstituted Ar_1;

X_2 is O or NR_5, where R_5 is selected from the group consisting of hydrogen, C_1-C_6 straight or branched alkyl and alkenyl;

R_4 is selected from the group consisting of phenyl, benzyl, C_1-C_5 straight or branched alkyl or alkenyl, and C_1-C_5 straight or branched alkyl or alkenyl substituted with phenyl; or pharmaceutically acceptable salts or hydrates thereof.

Another preferred embodiment of the invention is a neurotrophic compound having an affinity for FKBP-type immunophilins which inhibit the rotamase activity of the immunophilin.

Another preferred embodiment of the present invention is a method for treating a neurological disorder in an animal comprising administering a therapeutically effective amount of a compound having an affinity for FKBP-type immunophilins which inhibits the rotamase activity of the immunophilin.

Another preferred embodiment of the invention is a method of promoting neuronal regeneration and growth in mammals, comprising administering to a mammal an effective amount of a neurotrophic compound having an affinity for FKBP-type immunophilins which inhibits the rotamase activity of the immunophilin.

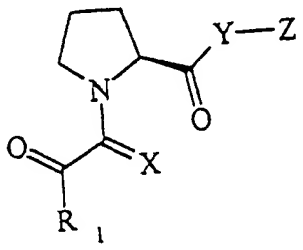
Yet another preferred embodiment of the invention is a method of preventing neurodegeneration in an animal comprising administering to an animal an effective amount of a neurotrophic compound having an affinity for FKBP-type immunophilins which inhibits rotamase activity of the immunophilin.

DETAILED DESCRIPTION OF THE INVENTION

The novel neurotrophic compounds of this invention are relatively small molecules in relation to other known compounds which bind to FKBP-type immunophilins, such as rapamycin, FK506, and cyclosporin.

The neurotrophic compounds of this invention have an affinity for the FK506 binding proteins such as FKBP-12. When the neurotrophic compounds of the invention are bound to the FKBP, they have been found

to unexpectedly inhibit the prolyl- peptidyl cis-trans isomerase activity, or rotamase activity of the binding protein and stimulate neurite growth, while not exhibiting an immunosuppressant effect. More particularly, this invention relates to a novel class of neurotrophic compounds represented by the formula:



I

where

R_1 is a C_1-C_9 straight or branched chain alkyl or alkenyl group optionally substituted with C_3-C_8 cycloalkyl, C_3 or C_5 cycloalkyl, C_5-C_7 cycloalkenyl, or Ar_1, where said alkyl, alkenyl, cycloalkyl or cycloalkenyl groups may be optionally substituted with C_1-C_4 alkyl, C_1-C_4 alkenyl, or hydroxy, and where Ar_1 is selected from the group consisting of 1-napthyl, 2-napthyl, 2-indolyl, 3-indolyl, 2-furyl, 3-furyl, 2- thiazolyl, 2-thienyl, 3-thienyl, 2-, 3-, or 4-pyridyl, or phenyl, having one to three substituents which are independently selected from the group

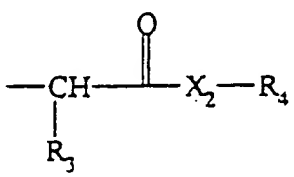
consisting of hydrogen, halo, hydroxyl, nitro, trifluoromethyl, C_1-C_5 straight or branched alkyl or alkenyl, C_1-C_4 alkoxy or C_1-C_4 alkenyloxy, phenoxy, benzyloxy, and amino;

X is oxygen, sulfur, methylene (CH_2), or H_2;

Y is oxygen or NR_2, where R_2 is hydrogen or C_1-C_6 alkyl; and

Z is a C_2-C_6 straight or branched chain alkyl or alkenyl, wherein the alkyl chain is substituted in one or more positions with Ar_1 as defined above, C_3-C_8 cycloalkyl, cycloalkyl connected by a C_1-C_6 straight or unbranched alkyl or alkenyl chain, or Ar_2 where Ar_2 is selected from the group consisting of 2-indolyl, 3-indolyl, 2-furyl, 3-furyl, 2- thiazolyl, 2-thienyl, 3-thienyl, 2-, 3-, or 4-pyridyl, or phenyl, having one to three substituents which are independently selected from the group consisting of hydrogen, halo, hydroxyl, nitro, trifluoromethyl, C_1-C_6 straight or branched alkyl or alkenyl, C_1-C_4 alkoxy or C1-C4 alkenyloxy, phenoxy, benzyloxy, and amino;

Z may also be the fragment:



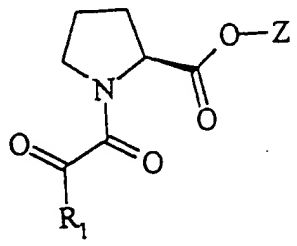
where

R_3 is selected from the group consisting of straight or branched alkyl C_1-C_8 optionally substituted with C_3-C_8 cycloalkyl, or Ar_1 as defined above, and unsubstituted Ar_1;

X_2 is O or NR_5, where R_5 is selected from the group consisting of hydrogen, C_1-C_6 straight or branched alkyl and alkenyl;

R_4 is selected from the group consisting of phenyl, benzyl, C_1-C_5 straight or branched alkyl or alkenyl, and C_1-C_5 straight or branched alkyl or alkenyl substituted with phenyl; or pharmaceutically acceptable salts or hydrates thereof.

Preferred compounds have the following formula:



II

where

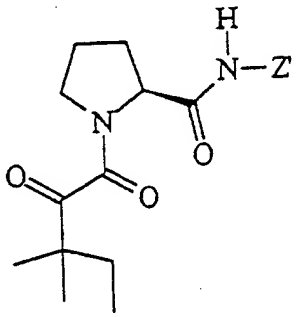
R_1 is a C_1-C_9 straight or branched chain alkyl or alkenyl group optionally substituted with C_3-C_8 cycloalkyl, C_3 or C_5 cycloalkyl, C_5-C_7

cycloalkenyl, or Ar_1, where said alkyl, alkenyl, cycloalkyl or cycloalkenyl groups may be optionally substituted with C_1-C_4 alkyl, C_1-C_4 alkenyl, or hydroxy, and where Ar_1 is selected from the group consisting of 1-napthyl, 2-napthyl, 2-indolyl, 3-indolyl, 2-furyl, 3-furyl, 2- thiazolyl, 2-thienyl, 3-thienyl, 2-, 3-, or 4-pyridyl, or phenyl, having one to three substituents which are independently selected from the group consisting of hydrogen, halo, hydroxyl, nitro, trifluoromethyl, C_1-C_6 straight or branched alkyl or alkenyl, C_1-C_4 alkoxy or C_1-C_4 alkenyloxy, phenoxy, benzyloxy, and amino;

Z is a C_2-C_6 straight or branched chain alkyl or alkenyl, wherein the alkyl chain is substituted in one or more positions with Ar_1 as defined above, C3-C8 cycloalkyl, cycloalkyl connected by a C_1-C_6 straight or unbranched alkyl or alkenyl chain, or Ar_2 where Ar_2 is selected from the group consisting of 2-indolyl, 3-indolyl, 2-furyl, 3-furyl, 2- thiazolyl, 2-thienyl, 3-thienyl, 2-, 3-, or 4-pyridyl, or phenyl, having one to three substituents which are independently selected from the group consisting of hydrogen, halo, hydroxyl, nitro, trifluoromethyl, C_1-C_6 straight or

branched alkyl or alkenyl, C_1-C_4 alkoxy or C_1-C_4 alkenyloxy, phenoxy, benzyloxy, and amino; or pharmaceutically acceptable salts or hydrates thereof.

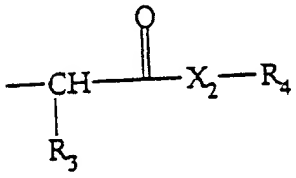
In another preferred embodiment novel compounds of this invention are represented by the formula:



III

where

Z' is the fragment:



where

R_3 is selected from the group consisting of straight or branched alkyl C_1-C_8 optionally substituted with C_3-C_8 cycloalkyl, or Ar_1 as defined above, or unsubstituted Ar_1;

X_2 is O or NR_5, where R_5 is selected from the group consisting of hydrogen, C_1-C_6 straight or branched alkyl and alkenyl;

R_4 is selected from the group consisting of phenyl, benzyl, C_1-C_5 straight or branched alkyl or alkenyl, and C_1-C_5 straight or branched alkyl or alkenyl substituted with phenyl; or pharmaceutically acceptable salts or hydrates thereof.

The compounds of this invention exist as stereoisomeric forms, either enantiomers or diastereoisomers. The stereochemistry at position 1 (Formula 1) is R or S, with S preferred. Included within the scope of the invention are the enantiomers, the racemic form, and diastereoisomeric mixtures. Enantiomers as well as diastereoisomers can be separated by methods known to those skilled in the art.

It is known that immunophilins such as FKBP preferentially recognize peptide substrates containing Xaa-Pro-Yaa motifs, where Xaa and Yaa are lipophilic amino acid residues. Schreiber et al. 1990 *J. Org. Chem.* 55, 4984-4986; Harrison and Stein, 1990 *Biochemistry*, 29, 3813-3816. Thus modified prolyl peptidomimetic compounds bearing lipophilic substituents should bind with high affinity to the hydrophobic core of the FKBP active site and inhibit its rotamase activity.

Preferred compounds of the invention include:

3-phenyl-1-propyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate,

3-phenyl-1-prop-2-(E)-enyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate,

3-(3,4,5-trimethoxyphenyl)-1-propyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate,

3-(3,4,5-trimethoxyphenyl)-1-prop-2-(E)-enyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate,

3-(4,5-dichlorophenyl)-1-propyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate,

3-(4,5-dichlorophenyl)-1-prop-2-(E)-enyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate,

3-(4,5-methylenedioxyphenyl)-1-propyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate,

3-(4,5-methylenedioxyphenyl)-1-prop-2-(E)-enyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate,

3-cyclohexyl-1-propyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate,

3-cyclohexyl-1-prop-2-(E)-enyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate,

(1R)-1,3-diphenyl-1-propyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate,

(1R)-1,3-diphenyl-1-prop-2-(E)-enyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate,

(1R)-1-cyclohexyl-3-phenyl-1-propyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate,

(1R)-1-cyclohexyl-3-phenyl-1-prop-2-(E)-enyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate,

(1R)-1-(4,5-dichlorophenyl)-3-phenyl-1-propyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate,

3-phenyl-1-propyl (2S)-1-(1,2-dioxo-2-cyclohexyl)ethyl-2-pyrrolidinecarboxylate,

3-phenyl-1-propyl (2S)-1-(1,2-dioxo-4-cyclohexyl)butyl-2-pyrrolidinecarboxylate,

3-phenyl-1-propyl (2S)-1-(1,2-dioxo-2-[2-furanyl])ethyl-2-pyrrolidinecarboxylate,

3-phenyl-1-propyl (2S)-1-(1,2-dioxo-2-[2-thienyl])ethyl-2-pyrrolidinecarboxylate,

3-phenyl-1-propyl (2S)-1-(1,2-dioxo-2-[2-thiazolyl])ethyl-2-pyrrolidinecarboxylate,

3-phenyl-1-propyl (2S)-1-(1,2-dioxo-2-phenyl)ethyl-2-pyrrolidinecarboxylate,

1,7-diphenyl-4-heptyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate,

3-Phenyl-1-propyl (2S)-1-(3,3-dimethyl-1,2-dioxo-4-hydroxybutyl)-2-pyrrolidinecarboxylate,

3-phenyl-1-propyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxamide,

1-[1-(3,3-dimethyl-1,2-dioxopentyl)-L-proline]-L-phenylalanine ethyl ester,

1-[1-(3,3-dimethyl-1,2-dioxopentyl)-L-proline]-L-leucine ethyl ester,

1-[1-(3,3-dimethyl-1,2-dioxopentyl)-L-proline]-L-phenylglycine ethyl ester,

1-[1-(3,3-dimethyl-1,2-dioxopentyl)-L-proline]-L-phenylalanine phenyl ester,

1-[1-(3,3-dimethyl-1,2-dioxopentyl)-L-proline]-L-phenylalanine benzyl ester, and

1-[1-(3,3-dimethyl-1,2-dioxopentyl)-L-proline]-L-isoleucine ethyl ester.

The compounds of the present invention can be used in the form of salts derived from inorganic or organic acids and bases. Included among such acid salts are the following: acetate, adipate, alginate, aspartate, benzoate, benzenesulfonate, bisulfate butyrate, citrate, camphorate, camphorsulfonate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, fumarate, glucoheptanoate, glycerophosphate, hemissulfate heptanoate, hexanoate, hydrochloride, hydrobromide, hydroiodide, 2-hydroxyethanesulfonate, lactate, maleate, methanesulfonate, 2-naphthalensulfonate, nicotinate, oxalate, pamoate, pectinate, propionate, succinate, tartrate, thiocyanate, tosylate and undecanoate. Base salts include ammonium salts, alkali metal salts such as sodium and potassium salts, alkaline earth metal salts such as calcium and magnesium salts, salt with organic bases such as dicyclohexylamine salts, N-methyl-D-glucamine, and salts with amino acids such as arginine, lysine, and so forth. Also, the basic

nitrogen-containing groups can be quarternized with such agents as lower alkyl halides, such as methyl, ethyl, propyl, and butyl chloride, bromides and iodides; dialkyl sulfates like dimethyl, diethyl, dibutyl and diamyl sulfates, long chain halides such as decyl, lauryl, myristyl and stearyl chlorides, bromides and iodides, aralkyl halides like benzyl and phenethyl bromides and others. Water or oil-soluble or dispersible products are thereby obtained.

The neurotrophic compounds of this invention can be periodically administered to a patient undergoing treatment for neurological disorders or for other reasons in which it is desirable to stimulate neuronal regeneration and growth, such as in various peripheral neuropathic and neurological disorders relating to neurodegeneration. The compounds of this invention can also be administered to mammals other than humans for treatment of various mammalian neurological disorders.

The novel compounds of the present invention are potent inhibitors of rotamase activity and possess an excellent degree of neurotrophic activity. This activity is useful in the stimulation of damaged neurons, the promotion of neuronal regeneration, the prevention of neurodegeneration, and in the treatment of several neurological disorders known to be associated with neuronal degeneration and peripheral neuropathies. The neurological disorders that may be treated include but are not limited to: trigeminal

neuralgia, glossopharyngeal neuralgia, Bell's Palsy, myasthenia gravis, muscular dystrophy, amyotrophic lateral sclerosis, progressive muscular atrophy, progressive bulbar inherited muscular atrophy, herniated, ruptured or prolapsed invertabrae disk syndromes, cervical spondylosis, plexus disorders, thoracic outlet destruction syndromes, peripheral neuropathic such as those caused by lead, dapsone, ticks, prophyria, or Gullain-Barré syndrome, Alzheimer's disease, and Parkinson's disease.

For these purposes the compounds of the present invention may be administered orally, parenterally, by inhalation spray, topically, rectally, nasally, buccally, vaginally or via an implanted reservoir in dosage formulations containing conventional non-toxic pharmaceutically-acceptable carriers, adjuvants and vehicles. The term parenteral as used herein includes subcutaneous, intravenous, intramuscular, intraperitoneally, intrathecally, intraventricularly, intrasternal and intracranial injection or infusion techniques.

To be effective therapeutically as central nervous system targets the immunophilin-drug complex should readily penetrate the blood-brain barrier when peripherally administered. Compounds of this invention which cannot penetrate the blood-brain barrier can be effetively administered by an intraventricular route.

The pharmaceutical compositions may be in the form of a sterile injectable preparation, for example as a sterile injectable aqueous or oleaginous suspension. This suspension may be formulated according to techniques know in the art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent, for example as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono- or diglycerides. Fatty acids such as oleic acid and its glyceride derivatives find use in the preparation of injectables, olive oil or castor oil, especially in their polyoxyethylated versions. These oil solutions or suspensions may also contain a long-chain alcohol diluent or dispersant.

The compounds may be administered orally in the form of capsules or tablets, for example, or as an aqueous suspension or solution. In the case of tablets for oral use, carriers which are commonly used include lactose and corn starch. Lubricating agents, such as magnesium stearate, are also typically added. For oral

administration in a capsule form, useful diluents include lactose and dried corn starch. When aqueous suspensions are required for oral use, the active ingredient is combined with emulsifying and suspending agents. If desired, certain sweetening and/or flavoring and/or coloring agents may be added.

The compounds of this invention may also be administered in the form of suppositories for rectal administration of the drug. These compositions can be prepared by mixing the drug with a suitable non-irritating excipient which is solid at room temperature but liquid at rectal temperature and therefore will melt in the rectum to release the drug. Such materials include cocoa butter, beeswax and polyethylene glycols.

The compounds of this invention may also be administered optically, especially when the conditions addressed for treatment involve areas or organs readily accessible by topical application, including neurological disorders of the eye, the skin, or the lower intestinal tract. Suitable topical formulations are readily prepared for each of these areas.

For ophthalmic use, the compounds can be formulated as micronized suspensions in isotonic, pH adjusted sterile saline, or, preferably, as solutions is isotonic, pH adjusted sterile saline, either with or without a preservative such as benzylalkonium chloride. Alternatively for the ophthalmic uses the compounds may be formulated in an ointment such as petrolatum.

For application topically to the skin, the compounds can be formulated in a suitable ointment containing the compound suspended or dissolved in, for example, a mixture with one or more of the following: mineral oil, liquid petrolatum, white petrolatum, propylene glycol, polyoxyethylene polyoxypropylene compound, emulsifying wax and water. Alternatively, the compounds can be formulated in a suitable lotion or cream containing the active compound suspended or dissolved in, for example, a mixture of one or more of the following: mineral oil, sorbitan monostearate, polysorbate 60, cetyl esters wax, cetearyl alcohol, 2-octyldodecanol, benzyl alcohol and water.

Topical application for the lower intestinal tract an be effected in a rectal suppository formulation (see above) or in a suitable enema formulation.

Dosage levels on the order of about .1mg to about 10,000 mg. of the active ingredient compound are useful in the treatment of the above conditions, with preferred levels of about 0.1mg to about 1,000 mg. The amount of active ingredient that may be combined with the carrier materials to produce a single dosage form will vary depending upon the host treated and the particular mode of administration.

It is understood, however, that a specific dose level for any particular patient will depend upon a variety of factors including the activity of the specific compound employed, the age, body weight,

general health, sex, diet, time of administration, rate of excretion, drug combination, and the severity of the particular disease being treated and form of administration.

The compounds can be administered with other neurotrophic agents such as neurotrophic growth factor (NGF), glial derived growth factor, brain derived growth factor, ciliary neurotrophic factor, and neurotropin-3. The dosage level of other neurotrophic drugs will depend upon the factors previously stated and the neurotrophic effectiveness of the drug combination.

K_i Test Procedure

Inhibition of the peptidyl-prolyl isomerase (rotamase) activity of the inventive compounds can be evaluated by known methods described in the literature (Harding, M.W. et al. *Nature* 341: 758-760 (1989); Holt et al. *J. Am. Chem. Soc.* 115: 9923-9938). These values are obtained as apparent K_i's and are presented for some of Examples 1-30 in Table I. The *cis-trans* isomerization of an alanine-proline bond in a model substrate, N-succinyl-Ala-Ala-Pro-Phe-*p*-nitroanilide, is monitored spectrophotometrically in a chymotrypsin-coupled assay, which releases *para*-nitroanilide from the *trans* form of the substrate. The inhibition of this reaction caused by the addition of different concentrations of inhibitor is determined, and the data is analyzed as a change in first-order rate constant as

a function of inhibitor concentration to yield the apparent K_i values.

In a plastic cuvette are added 950 mL of ice cold assay buffer (25 mM HEPES, pH 7.8, 100 mM NaCl), 10 mL of FKBP (2.5 mM in 10 mM Tris-Cl pH 7.5, 100 mM NaCl, 1 mM dithiothreitol), 25 mL of chymotrypsin (50 mg/ml in 1 mM HCl) and 10 mL of test compound at various concentrations in dimethyl sulfoxide. The reaction is initiated by the addition of 5 mL of substrate (succinyl-Ala-Phe-Pro-Phe-para-nitroanilide, 5 mg/mL in 2.35 mM LiCl in trifluoroethanol).

The absorbance at 390 nm versus time is monitored for 90 sec using a spectrophotometer and the rate constants are determined from the absorbance versus time data files.

The data for these experiments is presented in Table I.

TABLE I

FKBP ROTAMASE INHIBITION

Example	K_inM
4	42
5	125
6	200
7	65
8	2500
9	160
10	52
24	9000

In mammalian cells, FKBP-12 complexes with the inositol triphosphate receptor (IP_3R) and the ryanodine receptor (RyR). It is believed that the neurotrophic compounds of this invention disassociates FKBP-12 from these complexes causing the calcium channel to become "leaky" (Cameron et al., 1995). Calcium fluxes are involved in neurite extensions so that the IP_3R receptor and the ryanodine receptor might be involved in the neurotrophic effects of drugs. Since the drugs bind to the same site as FKBP-12 as the IP_3R receptor, one could assume that the drugs displace the channels from FKBP-12.

Chick Dorsal Root Ganglion Cultures and Neurite Outgrowth

Dorsal root ganglia were dissected from chick embryos of ten day gestation. Whole ganglion explants were cultured on thin layer Matrigel-coated 12 well plates with Liebovitz L15 plus high glucose media supplemented with 2mM glutamine and 10% fetal calf serum, and also containing 10 μM cytosine β-D arabinofuranoside (Ara C) at 37°C in an environment containing 5% CO_2. Twenty-four hours later, the DRGs were treated with various concentrations of nerve growth factor, immunophilin ligands or combinations of NFG plus drugs. Forty-eight hours after drug treatment, the ganglia were visualized under phase contrast or Hoffman Modulation contrast with a Zeiss Axiovert inverted microscope. Photomicrographs of the explants were made, and neurite outgrowth was quantitated. Neurites longer than the DRG diameter were counted as positive, with total number of neurites quantitated per each experimental condition. Three to four DRGs are cultured per well, and each treatment was performed in duplicate.

The data for these experiments are presented in Table II.

TABLE II

Neurite Outgrowth in Chick DRG

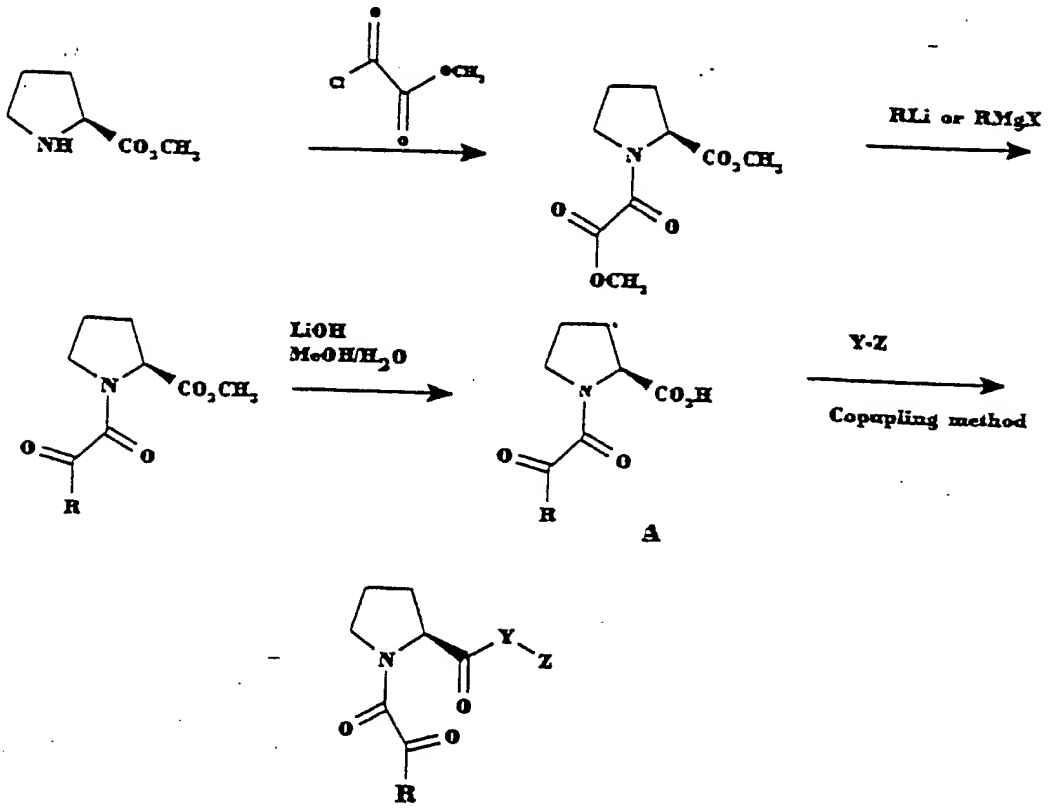
Example	ED_{50} (nM)
4	53
5	105
6	149
7	190
8	850
9	75
10	------
24	------

The following examples are illustrative of preferred embodiments of the invention and are not to be construed as limiting the invention thereto. All polymer molecular weights are mean average molecular weights. All percentages are based on the percent by weight of the final delivery system or formulation prepared unless otherwise indicated and all totals equal 100% by weight.

EXAMPLES

The inventive compounds may be prepared by a variety of synthetic sequences that utilize established chemical transformations. The general pathway to the present compounds is described in Scheme 1. N-glyoxylproline derivatives may be prepared by reacting

L-proline methyl ester with methyl oxalyl chloride as shown in Scheme I. The resulting oxamates may be reacted with a variety of carbon nucleophiles to obtain intermediates compounds. These intermediates are then reacted with a variety of alcohols, amides, or protected amino acid residues to obtain the propyl esters and amides of the invention.



Scheme I

EXAMPLE 1

Synthesis of methyl (2S)-1-(1,2-dioxo-2-methoxyethyl)-2-pyrrolidinecarboxylate.

A solution of L-proline methyl ester hydrochloride (3.08 g; 18.60 mmol) in dry methylene chloride was cooled to 0°C and treated with triethylamine (3.92 g; 38.74 mmol; 2.1 eq). After stirring the formed slurry under a nitrogen atmosphere for 15 min, a solution of methyl oxalyl chloride (3.20 g; 26.12 mmol) in methylene chloride (45 mL) was added dropwise. The resulting mixture was stirred at 0°C for 1.5 hr. After filtering to remove solids, the organic phase was washed with water, dried over $MgSO_4$ and concentrated. The crude residue was purified on a silica gel column, eluting with 50% ethyl acetate in hexane, to obtain 3.52 g (88%) of the product as a reddish oil. Mixture of cis-trans amide rotamers; data for trans rotamer given. ^1H NMR ($CDCl_3$): d 1.93 (dm, 2H); 2.17 (m, 2H); 3.62 (m, 2H); 3.71 (s, 3H); 3.79, 3.84 (s, 3H total); 4.86 (dd, 1H, J = 8.4, 3.3).

EXAMPLE 2

General procedure for the synthesis of pyrrolidinyl alkyl oxamates. Exemplified for methyl (2S)-1-(1,2-dioxo-3,3-dimethylpentyl)-2-pyrrolidinecarboxylate.

A solution of methyl (2S)-1-(1,2-dioxo-2-methoxyethyl)-2-pyrrolidinecarboxylate (2.35 g; 10.90

mmol) in 30 mL of tetrahydrofuran (THF) was cooled to -78°C and treated with 14.2 mL of a 1.0 M solution of 1,1-dimethylpropylmagnesium chloride in THF. After stirring the resulting homogeneous mixture at -78°C for three hours, the mixture was poured into saturated ammonium chloride (100 mL) and extracted into ethyl acetate. The organic phase was washed with water, dried, and concentrated, and the crude material obtained upon removal of the solvent was purified on a silica gel column, eluting with 25% ethyl acetate in hexane, to obtain 2.10 g (75%) of the oxamate as a colorless oil. ^{1}H NMR ($CDCl_3$): d 0.88 (t, 3H); 1.22, 1.26 (s, 3H each); 1.75 (dm, 2H); 1.87-2.10 (m, 3H); 2.23 (m, 1H); 3.54 (m, 2H); 3.75 (s, 3H); 4.52 (dm, 1H, J = 8.4, 3.4).

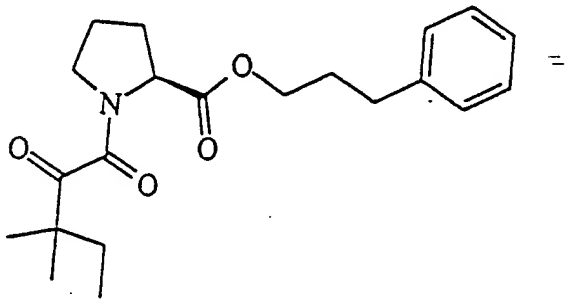
EXAMPLE 3

General procedure for the preparation of pyrrolidine carboxylic acids. Exemplified for (2S)-1-(1,2-dioxo-3,3-dimethylpentyl)-2-pyrrolidinecarboxylic acid.

A mixture of methyl (2S)-1-(1,2-dioxo-3,3-dimethylpentyl)-2-pyrrolidinecarboxylate (2.10 g; 8.23 mmol), 1 N LiOH (15 mL), and methanol (50 mL) was stirred at 0°C for 30 min and at room temperature overnight. The mixture was acidified to pH 1 with 1 N HCl, diluted with water, and extracted into 100 mL of methylene chloride. The organic extract was washed with

brine and concentrated to deliver 1.73 g (87%) of snow-white solid which did not require further purification. ^{1}H NMR ($CDCl_3$): d 0.87 (t, 3H); 1.22, 1.25 (s, 3H each); 1.77 (dm, 2H); 2.02 (m, 2H); 2.17 (m, 1H); 2.25 (m, 1H); 3.53 (dd, 2H, J = 10.4, 7.3); 4.55 (dd, 1H, J = 8.6, 4.1).

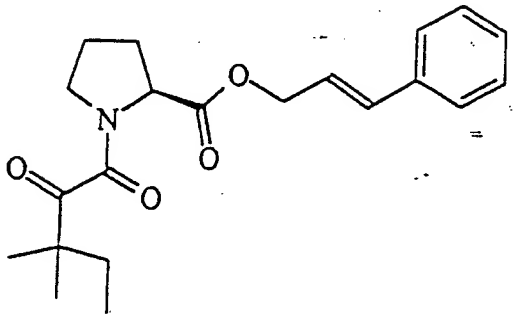
EXAMPLE 4



General procedure for the synthesis of prolyl esters. Exemplified for 3-phenyl-1-propyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate. A mixture of (2S)-1-(1,2-dioxo-3,3-dimethylpentyl)-2-pyrrolidine-carboxylic acid (600 mg; 2.49 mmol), 3-phenyl-1-propanol (508 mg; 3.73 mmol), dicyclohexylcarbodiimide (822 mg; 3.98 mmol), camphorsulphonic acid (190 mg; 0.8 mmol) and 4-dimethylaminopyridine (100 mg; 0.8 mmol) in methylene chloride (20 mL) was stirred overnight under a nitrogen atmosphere. The reaction mixture was filtered through Celite to remove solids and concentrated in vacuo, and the crude material was purified on a flash column (25% ethyl acetate in hexane) to obtain 720 mg (80%) of the

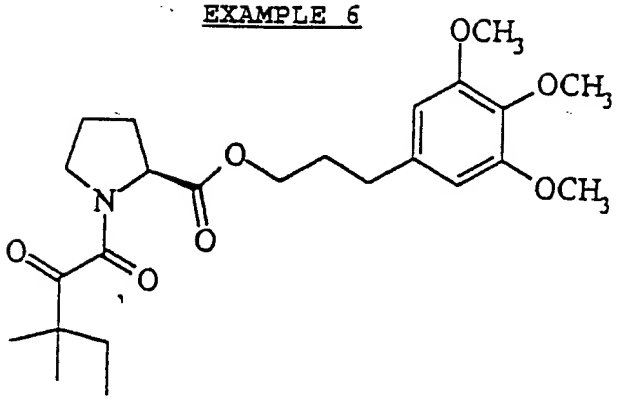
product as a colorless oil. ^{1}H NMR ($CDCl_3$): d 0.84 (t, 3H); 1.19 (s, 3H); 1.23 (s, 3H); 1.70 (dm, 2H); 1.98 (m, 5H); 2.22 (m, 1H); 2.64 (m, 2H); 3.47 (m, 2H); 4.14 (m, 2H); 4.51 (d, 1H); 7.16 (m, 3H); 7.26 (m, 2H).

EXAMPLE 5



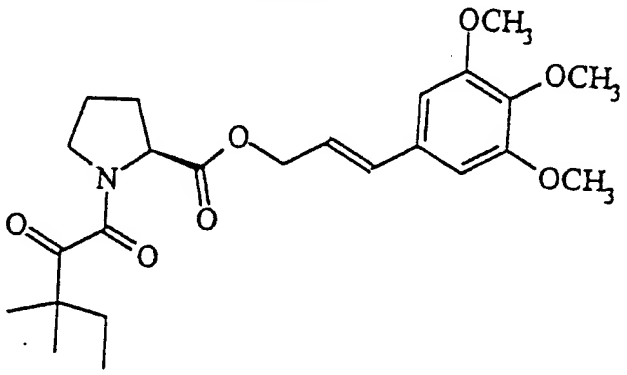
3-Phenyl-1-prop-2-(E)-enyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate, 80%, ^{1}H NMR (360 Mhz, $CDCl_3$): d 0.86 (t, 3H); 1.21 (s, 3H); 1.25 (s, 3H); 1.54-2.10 (m, 5H); 2.10-2.37 (m, 1H); 3.52-3.55 (m, 2H); 4.56 (dd, 1H, J = 3.8, 8.9); 4.78-4.83 (m, 2H); 6.27 (m, 1H); 6.67 (dd, 1H, J = 15.9); 7.13-7.50 (m, 5H). This compound was prepared by the method of Example 3 from (2S)-1-(1,2-dioxo-3,3-dimethylpentyl)-2-pyrrolidine-carboxylic acid.

EXAMPLE 6



3-(3,4,5-Trimethoxyphenyl)-1-propyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate, 61%, ^{1}H NMR ($CDCl_3$): d 0.84 (t, 3H); 1.15 (s, 3H); 1.24 (s, 3H); 1.71 (dm, 2H); 1.98 (m, 5H); 2.24 (m, 1H); 2.63 (m, 2H); 3.51 (t, 2H); 3.79 (s, 3H); 3.83 (s, 3H); 4.14 (m, 2H); 4.52 (m, 1H); 6.36 (s, 2H). This compound was prepared by the method of Example 3 from (2S)-1-(1,2-dioxo-3,3-dimethylpentyl)-2-pyrrolidine-carboxylic acid.

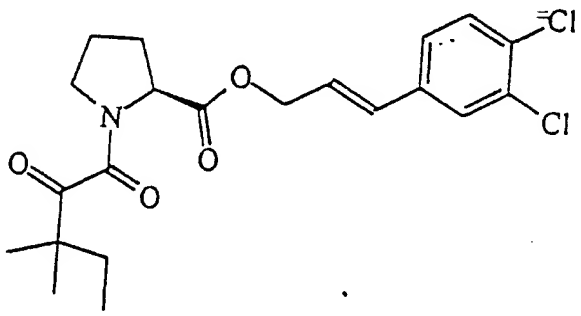
EXAMPLE 7



3-(3,4,5-Trimethoxyphenyl)-1-prop-2-(E)-enyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate, 66%, ^{1}H NMR ($CDCl_3$): d 0.85 (t,

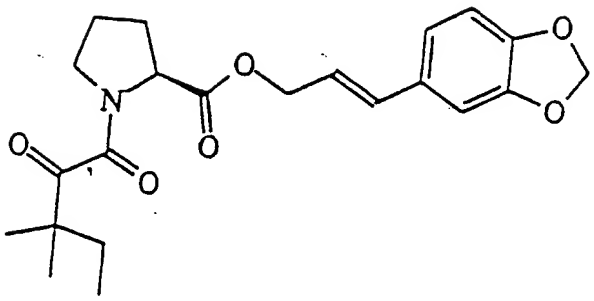
3H); 1.22 (s, 3H); 1.25 (s, 3H); 1.50-2.11 (m, 5H); 2.11-2.40 (m, 1H); 3.55 (m, 2H); 3.85 (s, 3H); 3.88 (s, 6H); 4.56 (dd, 1H); 4.81 (m, 2H); 6.22 (m, 1H); 6.58 (d, 1H, J = 16); 6.63 (s, 2H). This compound was prepared by the method of Example 3 from (2S)-1-(1,2-dioxo-3,3-dimethylpentyl)-2-pyrrolidine-carboxylic acid.

EXAMPLE 8



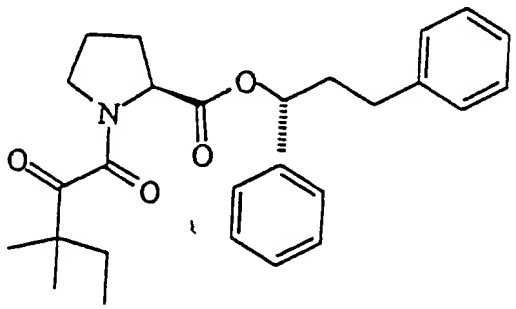
3-,4,5-Dichlorophenyl)-1-prop-2-(E)-enyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate, 70%, ^{1}H NMR ($CDCl_3$): d 0.85 (t, 3H); 1.21 (s, 3H); 1.25 (s, 3H); 1.51-1.87 (m, 2H); 1.87-2.39 (m, 4H); 3.51-3.57 (m, 2H); 4.50-4.61 (dd, 1H, J = 3.4, 8.6); 4.80 (d, 2H, J = 6.0); 6.20-6.34 (m, 1H); 6.50-6.66 (d, 1H, J = 16); 7.13-7.24 (dd, 1H, J = 1.8, 8.3); 7.39 (d, 1H, J = 8.3); 7.47 (s, 1H). This compound was prepared by the method of Example 3 from (2S)-1-(1,2-dioxo-3,3-dimethylpentyl)-2-pyrrolidine-carboxylic acid.

EXAMPLE 9



3-(4,5-Methylenedioxyphenyl)-1-prop-2-(E)-enyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate, 82%, ^{1}H NMR (360 MHz, $CDCl_3$): d 0.86 (t,3H); 1.22 (s, 3H); 1.25 (s, 3H); 1.60-2.10 (m, 5H); 3.36-3.79 (m, 2H); 4.53 (dd, 1H, J = 3.8, 8.6); 4.61-4.89 (m, 2H); 5.96 (s, 2H); 6.10 (m, 1H); 6.57 (dd, 1H, J = 6.2, 15.8); 6.75 (d, 1H, J = 8.0); 6.83 (dd, 1H, J = 1.3, 8.0); 6.93 (s, 1H). This compound was prepared by the method of Example 3 from (2S)-1-(1,2-dioxo-3,3-dimethylpentyl)-2-pyrrolidine-carboxylic acid.

EXAMPLE 10

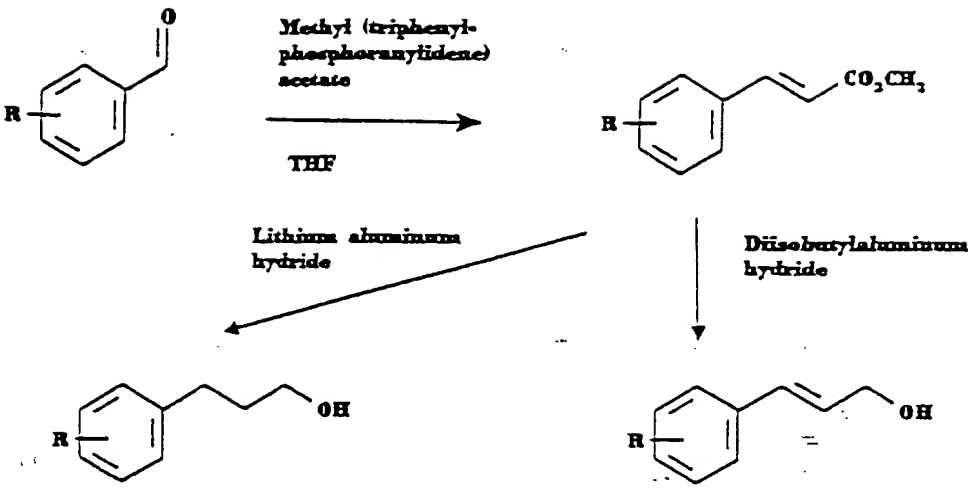


(1R)-1,3-Diphenyl-1-propyl (2S)-1-(3,3-dimethyl-

1,2-dioxopentyl)-2-pyrrolidinecarboxylate, 90%, ^{1}H NMR (360 MHz, $CDCl_3$): d 0.85 (t, 3H); 1.20 (s, 3H); 1.23 (s, 3H); 1.49-2.39 (m, 7H); 2.46-2.86 (m, 2H); 3.25-3.80 (m, 2H); 4.42-4.82 (m, 1H); 5.82 (td, 1H, J = 1.8, 6.7); 7.05-7.21 (m, 3H); 7.21-7.46 (m, 7H). This compound was prepared by the method of Example 3 from (2S)-1-(1,2-dioxo-3,3-dimethylpentyl)-2-pyrrolidine-carboxylic acid.

EXAMPLE 11

The requisite substituted alcohols may be prepared by a number of methods known to those skilled in the art of organic synthesis. As described in Scheme II, substituted benzaldehydes may be homologated to phenyl propanols by reaction with methyl (triphenylphosphoranylidene)- acetate to provide a variety of *trans*-cinnamates; these latter may be reduced to the saturated alcohols by reaction with excess lithium aluminum hydride, or sequentially by reduction of the double bond by catalytic hydrogenation and reduction of the saturated ester by appropriate reducing agents. Alternatively, the *trans*-cinnamates may be reduced to (E)-allylic alcohols by the use of diisobutylaluminum hydride.



Scheme II

Longer chain alcohols may be prepared by homologation of benzylic and higher aldehydes. Alternatively, these aldehydes may be prepared by conversion of the corresponding phenylacetic and higher acids, and phenethyl and higher alcohols.

EXAMPLE 12

General procedure for the synthesis of acrylic esters, exemplified for methyl (3,3,5-trimethoxy)-*trans*-cinnamate.

A solution of 3,4,5-trimethoxybenzaldehyde (5.0 g; 25.48 mmol) and methyl (triphenyl-phosphoranylidene)acetate (10.0 g; 29.91 mmol) in tetrahydrofuran (250 mL) was refluxed overnight. After cooling, the reaction mixture was diluted with 200 mL of ethyl acetate and washed with 2 x 200 mL of water, dried, and concentrated in vacuo. The crude residue was chromatographed on a silica gel column, eluting with

25% ethyl acetate in hexane, to obtain 5.63 g (88%) of the cinnamate as a white crystalline solid, ^{1}H NMR (300 Mhz; $CDCl_3$): d 3.78 (s, 3H); 3.85 (s, 6H); 6.32 (d, 1H, J = 16); 6.72 (s, 2H); 7.59 (d, 1H, J = 16).

EXAMPLE 13

Methyl (4,5-dichloro)-*trans*-cinnamate, 80%, ^{1}H NMR (300 Mhz; $CDCl_3$): d 3.79 (s, 3H); 6.40 (d, 1H, J = 16.8); 7.32 (dd, 1H, J = 1.5, 8.1); 7.44 (d, 1H, J = 8.1); 7.56 (d, 1H, J = 16); 7.58 (s, 1H). This compound was prepared by the method of Example 11 from 3,4,5-trimethoxybenzaldehyde.

EXAMPLE 14

Methyl (4,5-methylenedioxy)-*trans*-cinnamate, 74%, ^{1}H NMR (360 Mhz; $CDCl_3$): d 3.79 (s, 3H); 6.01 (s, 2H); 6.26 (d, 1H, J = 16); 6.81 (d, 1H, J = 7.9); 7.00 (d, 1H, J = 8.2); 7.03 (s, 1H); 7.60 (d, 1H, J = 16). This compound was prepared by the method of Example 11 from 3,4,5-trimethoxybenzaldehyde.

EXAMPLE 15

Methyl (2-cyclohexyl)-(E)-acrylate, 80%, ^{1}H NMR (360 Mhz; $CDCl_3$): d 1.12-1.43 (m, 5H); 1.52-1.87 (m, 5H); 2.12 (m, 1H); 3.71 (s, 3H); 5.77 (dd, 1H, J = 1.2, 15.8); 6.92 (dd, 1H, J = 6.8, 15.8). This compound was prepared by the method of Example 11 from 3,4,5-trimethoxybenzaldehyde.

EXAMPLE 16

General procedure for the synthesis of saturated alcohols from acrylic esters. Exemplified for (3,4,5-

trimethoxy) phenylpropanol.

A solution of methyl (3,3,5-trimethoxy)-*trans*-cinnamate (1.81 g; 7.17 mmol) in tetrahydrofuran (30 mL) was added in a dropwise manner to a solution of lithium aluminum hydride (14 mmol) in THF (35 mL), with stirring and under an argon atmosphere. After the addition was complete, the mixture was heated to 75°C for 4 hours. After cooling, it was quenched by the careful addition of 15 mL of 2N NaOH followed by 50 mL of water. The resulting mixture was filtered through Celite to remove solids, and the filter cake was washed with ethyl acetate. The combined organic fractions were washed with water, dried, concentrated in vacuo, and purified on a silica gel column, eluting with ethyl acetate to obtain 0.86 g (53%) of the alcohol as a clear oil, ^{1}H NMR (300 Mhz; $CDCl_3$): d 1.23 (br, 1H); 1.87 (m, 2H); 2.61 (t, 2H, J = 7.1); 3.66 (t, 2H); 3.80 (s, 3H); 3.83 (s, 6H); 6.40 (s, 2H).

EXAMPLE 17

General procedure for the synthesis of trans-allylic alcohols from acrylic esters. Exemplified for (3,4,5-trimethoxy)phenylprop-2-(E)-enol.

A solution of methyl (3,3,5-trimethoxy)-*trans*-cinnamate (1.35 g; 5.35 mmol) in toluene (25 mL) was cooled to -10°C and treated with a solution of diisobutylaluminum hydride in toluene (11.25 mL of a 1.0 M solution; 11.25 mmol). The reaction mixture was stirred for 3 hrs at 0°C and then quenched with 3 mL of

methanol followed by 1 N HCl until the pH was 1. The reaction mixture was extracted into ethyl acetate and the organic phase was washed with water, dried and concentrated. Purification on a silica gel column eluting with 25% ethyl acetate in hexane furnished 0.96 g (80%) of a thick oil, ^{1}H NMR (360 Mhz; $CDCl_3$): d 3.85 (s, 3H); 3.87 (s, 6H); 4.32 (d, 2H, J = 5.6); 6.29 (dt, 1H, J = 15.8, 5.7), 6.54 (d, 1H, J = 15.8); 6.61 (s, 2H).

EXAMPLE 18

(4,5-dichloro)phenylprop-2-(E)-enol, 89%, ^{1}H NMR (360 Mhz; $CDCl_3$): d 1.55 (s, 1H); 4.34 (d, 2H, J = 4.4); 6.36 (dt, 1H, J = 15.9, 5.3); 6.54 (d, 1H, J = 15.9); 7.20 (dd, 1H, J = 8.3, 1.7); 7.38 (d, 1H, J = 8.3); 7.45 (d, 1H, J = 1.6). This compound was prepared by the method of Example 16 from (3,4,5-trimethoxy)-trans-cinnamate.

EXAMPLE 19

(4,5-methylenedioxy)phenylprop-2-(E)-enol, 80%, ^{1}H NMR (360 Mhz; $CDCl_3$): d 1.59 (br, 1H); 4.29 (br, 2H); 5.96 (s, 2H); 6.20 (dt, 1H, J = 15.8, 5.9); 6.52 (d, 1H, J = 15.8); 6.76 (d, 1H, J = 8.0); 6.82 (dd, 1H, J = 8.0, 1.2); 6.93 (d, 1H, J = 1.2). This compound was prepared by the method of Example 16 from (3,4,5-trimethoxy)-trans-cinnamate.

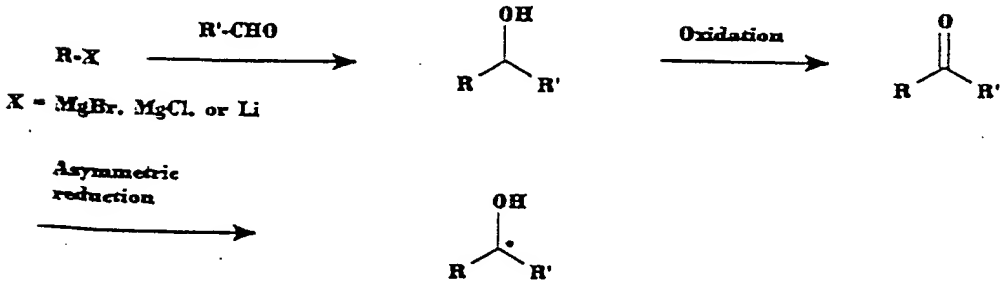
EXAMPLE 20

Phenylprop-2-(E)-enol, 85%, ^{1}H NMR (360 Mhz; $CDCl_3$): d 1.72 (br, 1H); 4.31 (d, 2H, J = 5.7); 6.36

(dt, 1H, J = 15.9, 5.7); 6.61 (d, 1H, J = 15.9); 7.02-7.55 (m, 5H). This compound was prepared by the method of Example 16 from (3,4,5-tri-methoxy)-trans-cinnamate.

EXAMPLE 21

Alcohols containing a substituent at the 1-position of the side chain may be conveniently prepared by addition of appropriate nucleophiles to aldehydes, as described in Scheme III. In cases where optically active substituted alcohols are desired, the racemic alcohols may be oxidized to prochiral ketones and subjected to asymmetric reduction by one of several methods well known to those skilled in the art.



Scheme III

EXAMPLE 22

General procedure for the preparation of 1-substituted alkanols, exemplified for the synthesis of 1,3-diphenylpropanol.

A solution of 2-(bromoethyl)benzene (17.45 g; 94.3 mmol) in 50 mL of dry diethyl ether was added dropwise, under a nitrogen atmosphere, to a stirred slurry of magnesium turnings (2.50 g; 102.8 mmol) in 50 mL of ether. The mixture was initially heated with a heat gun until reflux had become self-sustaining. After the addition was complete, the mixture was heated externally for 30 min to maintain reflux. A solution of 10.01 g (94.3 mmol) of benzaldehyde in 20 mL of ether was then added dropwise, and reflux was continued for 30 min. After cooling, the reaction mixture was poured into 150 mL of saturated ammonium chloride and extracted into ethyl acetate. The crude material obtained upon removal of the solvent was purified on a flash column, eluting with 5% ethyl acetate/hexane to 20% ethyl acetate, to obtain 13.73 g (69%) of the alkanol as a light yellow oil, ^{1}H NMR (360 Mhz; $CDCl_3$): d 1.93-2.30 (m, 3H); 2.70-2.90 (m, 2H); 4.72 (br, 1H); 7.19-7.27 (m, 3H); 7.27-7.36 (m, 3H); 7.36-7.47 (m, 4H).

EXAMPLE 23

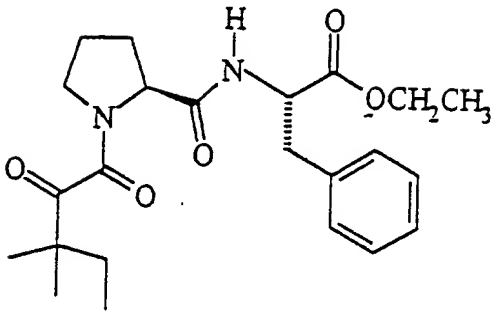
General procedure for conversion of racemic 1-substituted alkanols to optically active 1-substituted alkanols via prochiral ketones. Exemplified for (1R)-1,3-diphenyl-1-propanol.

A solution of racemic 1,3-diphenyl-1-propanol (1.26 g; 5.94 mmol) was dissolved in 10 mL of acetone, and Jones reagent was added until persistence of the

orange color. After stirring for 30 min, the reaction was quenched by adding 2 mL of 2-propanol. The solvent was decanted away from the precipitated solids, which were washed with ethyl acetate. The combined organic fractions were washed with 2 x 20 mL of water, dried and concentrated. The crude product was filtered through a plug of silica gel, eluting with 25% ethyl acetate/hexane, to obtain 1.07 g (86%) of 1,3-diphenylpropanone as a white crystalline solid, ^{1}H NMR (360 Mhz; $CDCl_3$): d 3.09 (t, 2H, J = 8.1); 3.33 (t, 2H, J = 8.1); 7.29 (m, 5H); 7.49 (m, 3H); 7.98 (m, 2H).

A solution of 1,3-diphenylpropanone (1.07 g; 5.09 mmol) in tetrahydrofuran (10 mL) was cooled to -23°C and treated with an asymmetric reducing agent, (+)-B-chlorodiisopinocampheyl-borane (1.80 g; 5.60 mmol) in 20 mL THF, and the resulting solution was allowed to stand overnight at -23°C. After evaporating to dryness, the residue was treated with ether (65 mL) and diethanolamine (1.0 g) and stirred for 3 hrs. The mixture was then filtered to remove solids and concentrated, and the residue was purified using gradient elution (5% ethyl acetate/hexane to 10% ethyl acetate) on a silica gel column to obtain 660 mg (61%) of (1R)-1,3-diphenyl-1-propanol as a crystalline white solid, ^{1}H NMR (360 Mhz; $CDCl_3$): d 1.95-2.15 (m, 3H); 2.59-2.78 (m, 2H); 4.65 (dd, 1H, J = 5.4, 7.8); 7.14-7.35 (m, 10H).

EXAMPLE 24



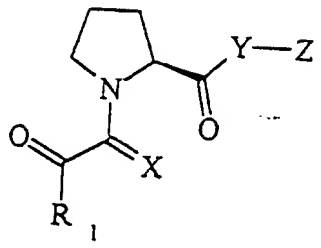
General procedure for the synthesis of prolyl dipeptides, exemplified for 1-[1-(3,3-dimethyl-1,2-dioxopentyl)-L-proline]-L-phenylalanine ethyl ester.

A mixture of (2S)-1-(1,2-dioxo-3,3-dimethylpentyl)-2-pyrrolidinecarboxylic acid (1.17 g; 4.85 mmol), L-phenylalanine ethyl ester hydrochloride (1.23 g; 5.33 mmol), dicyclohexylcarbo- diimide (1.10 g; 5.33 mmol) and 4-dimethylaminopyridine (60 mg (4.85 mmol) in methylene chloride (25 mL) was treated with triethylamine (1 mL; 726 mg; 7.17 mmol) and stirred overnight. The mixture was filtered through Celite to remove solids and concentrated, and the crude material from removal of the solvent was purified on a silica gel column eluting with 30% ethyl acetate/hexane to obtain 2.02 g of 1-[1-(3,3-dimethyl-1,2-dioxopentyl)-L-proline]-L-phenylalanine ethyl ester, 100%, ^{1}H NMR (360 MHz, $CDCl_3$): d 0.87 (t, 3H); 1.16-1.28 (m, 9H); 1.58-1.91 (m, 5H); 2.33 (m, 1H); 3.07-3.20 (m, 2H); 3.38-3.41 (m, 2H); 4.11-4.18 (m, 4H); 4.55 (d, 1H, J = 6.5); 4.78-4.80 (m, 1H); 7.15 (br d, 1H); 7.19 (m, 5H).

The invention being thus described, it will be obvious that the same may be varied in many ways. Such variations are not to be regarded as a departure from the spirit and scope of the invention and all such modification are intended to be included within the scope of the following claims.

What is claimed is:

1. A neurotrophic compound of the formula:



where

R_1 is a C_1-C_9 straight or branched chain alkyl or alkenyl group optionally substituted with C_3-C_9 cycloalkyl, C_3 or C_5 cycloalkyl, C_5-C_7 cycloalkenyl, or Ar_1, where said alkyl, alkenyl, cycloalkyl or cycloalkenyl groups may be optionally substituted with C_1-C_4 alkyl, C_1-C_4 alkenyl, or hydroxy, and where Ar_1 is selected from the group consisting of 1-napthyl, 2-napthyl, 2-indolyl, 3-indolyl, 2-furyl, 3-furyl, 2-thienyl, 3-thienyl, 2-, 3-, or 4-pyridyl, or phenyl, having one to three substituents which are independently selected from the group consisting of hydrogen, halo, hydroxyl, nitro, trifluoromethyl, C_1-C_6 straight or branched alkyl or alkenyl, C_1-C_4 alkoxy or C_1-C_4

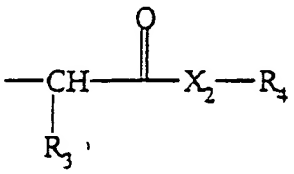
alkenyloxy, phenoxy, benzyloxy, and amino;

X is oxygen, sulfur, methylene (CH_2), or H_2;

Y is oxygen or NR_2, where R_2 is hydrogen or C_1-C_6 alkyl; and

Z is a C2-C6 straight or branched chain alkyl or alkenyl, wherein the alkyl chain is substituted in one or more positions with Ar_1 as defined above, C_3-C_8 cycloalkyl, cycloalkyl connected by a C_1-C_6 straight or unbranched alkyl or alkenyl chain, or Ar_2 where Ar_2 is selected from the group consisting of 2-indolyl, 3-indolyl, 2-furyl, 3-furyl, 2-thiazolyl, 2-thienyl, 3-thienyl, 2-, 3-, or 4-pyridyl, or phenyl, having one to three substituents which are independently selected from the group consisting of hydrogen, halo, hydroxyl, nitro, trifluoromethyl, C_1-C_6 straight or branched alkyl or alkenyl, C_1-C_4 alkoxy or C_1-C_4 alkenyloxy, phenoxy, benzyloxy, and amino;

Z may also be the fragment:



where

R_3 is selected from the group consisiting of straight or branched alkyl C_1-C_8 optionally substituted with C_3-C_8 cycloalkyl, or Ar_1 as defined above;

X_2 is O or NR_5, where R_5 is selected from the group consisting of hydrogen, C_1-C_6 straight or branched alkyl and alkenyl;

R_4 is selected from the group consisting of phenyl, benzyl, C_1-C_5 straight or branched alkyl or alkenyl, and C_1-C_5 straight or branched alkyl or alkenyl substituted with phenyl; or pharmaceutically acceptable salts or hydrates thereof.

2. The neurotrophic compound of claim 1, which has an affinity for FKBP-type immunophilins.

3. The neurotrophic compound of claim 2, where the FKBP-type immunophilin is FKBP-12.

4. The neurotrophic compound of claim 1, capable of inhibiting rotamase activity.

5. The neurotrophic compound of claim 1, where Z and R_1 are lipophilic groups.

6. The neurotrophic compound according to claim 1 that is selected from the group consisting of

3-phenyl-1-propyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate,

3-phenyl-1-prop-2-(E)-enyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate,

3-(3,4,5-trimethoxyphenyl)-1-propyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate,

3-(3,4,5-trimethoxyphenyl)-1-prop-2-(E)-enyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate,

3-(4,5-dichlorophenyl)-1-propyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate,

3-(4,5-dichlorophenyl)-1-prop-2-(E)-enyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate,

3-(4,5-methylenedioxyphenyl)-1-propyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate,

3-(4,5-methylenedioxyphenyl)-1-prop-2-(E)-enyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate,

3-cyclohexyl-1-propyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate,

3-cyclohexyl-1-prop-2-(E)-enyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate,

(1R)-1,3-diphenyl-1-propyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate,

(1R)-1,3-diphenyl-1-prop-2-(E)-enyl (2S)-1-(3,3-

dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate,

(1R)-1-cyclohexyl-3-phenyl-1-propyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate,

(1R)-1-cyclohexyl-3-phenyl-1-prop-2-(E)-enyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate,

(1R)-1-(4,5-dichlorophenyl)-3-phenyl-1-propyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate,

3-phenyl-1-propyl (2S)-1-(1,2-dioxo-2-cyclohexyl)ethyl-2-pyrrolidinecarboxylate,

3-phenyl-1-propyl (2S)-1-(1,2-dioxo-4-cyclohexyl)butyl-2-pyrrolidinecarboxylate,

3-phenyl-1-propyl (2S)-1-(1,2-dioxo-2-[2-furanyl])ethyl-2-pyrrolidinecarboxylate,

3-phenyl-1-propyl (2S)-1-(1,2-dioxo-2-[2-thienyl])ethyl-2-pyrrolidinecarboxylate,

3-phenyl-1-propyl (2S)-1-(1,2-dioxo-2-[2-thiazolyl])ethyl-2-pyrrolidinecarboxylate,

3-phenyl-1-propyl (2S)-1-(1,2-dioxo-2-phenyl)ethyl-2-pyrrolidinecarboxylate,

1,7-diphenyl-4-heptyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate,

3-Phenyl-1-propyl (2S)-1-(3,3-dimethyl-1,2-dioxo-4-hydroxybutyl)-2-pyrrolidinecarboxylate,

3-phenyl-1-propyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxamide,

1-[1-(3,3-dimethyl-1,2-dioxopentyl)-L-proline]-L-

phenylalanine ethyl ester,

1-[1-(3,3-dimethyl-1,2-dioxopentyl)-L-proline]-L-leucine ethyl ester,

1-[1-(3,3-dimethyl-1,2-dioxopentyl)-L-proline]-L-phenylglycine ethyl ester,

1-[1-(3,3-dimethyl-1,2-dioxopentyl)-L-proline]-L-phenylalanine phenyl ester,

1-[1-(3,3-dimethyl-1,2-dioxopentyl)-L-proline]-L-phenylalanine benzyl ester, and

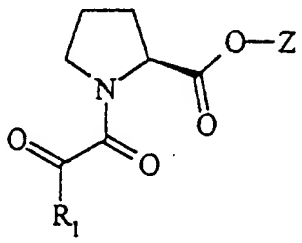
1-[1-(3,3-dimethyl-1,2-dioxopentyl)-L-proline]-L-isoleucine ethyl ester.

7. A pharmaceutical composition comprising a neurotrophically effective amouht of the compound of claim 1 and a pharmaceutically acceptable carrier.

8. A method of stimulating growth of damaged peripheral nerves, which comprises:

administering to damaged peripheral nerves the neurotrophic compound of claim 1 in sufficient amounts to stimulate the growth of said nerves.

9. A neurotrophic compound of the formula:



where

R_1 is a C_1-C_9 straight or branched chain alkyl or alkenyl group optionally substituted with C_3-C_9 cycloalkyl, C_3 or C_5 cycloalkyl, C_5-C_7 cycloalkenyl, or Ar_1, where said alkyl, alkenyl, cycloalkyl or cycloalkenyl groups may be optionally substituted with C_1-C_4 alkyl, C_1-C_4 alkenyl, or hydroxy, and where Ar_1 is selected from the group consisting of 1-napthyl, 2-napthyl, 2-indolyl, 3-indolyl, 2-furyl, 3-furyl, 2-thiazolyl, 2-thienyl, 3-thienyl, 2-, 3-, or 4-pyridyl, or phenyl, having one to three substituents which are independently selected from the group consisting of hydrogen, halo, hydroxyl, nitro, trifluoromethyl, C_1-C_6 straight or branched alkyl or alkenyl, C_1-C_4 alkoxy or C_1-C_4 alkenyloxy, phenoxy, benzyloxy, and amino;

Z is a C_2-C_6 straight or branched chain alkyl or alkenyl, wherein the alkyl chain is substituted in one or more positions with Ar_1 as defined above, C_3-C_8 cycloalkyl, cycloalkyl connected by a C_1-C_6 straight or unbranched alkyl or alkenyl chain, or Ar_2 where Ar_2 is selected from the group consisting of 2-indolyl, 3-indolyl, 2-furyl, 3-furyl, 2-thiazolyl, 2-thienyl, 3-thienyl, 2-, 3-, or 4-pyridyl, or phenyl,

having one to three substituents which are independently selected from the group consisting of hydrogen, halo, hydroxyl, nitro, trifluoromethyl, C_1-C_6 straight or branched alkyl or alkenyl, C_1-C_4 alkoxy or C_1-C_4 alkenyloxy, phenoxy, benzyloxy, and amino; or pharmaceutically acceptable salts or hydrates thereof.

10. The neurotrophic compound of claim 9, wherein R_1 is selected from the group consisting of C_1-C_9 straight or branched chain alkyl, 2-cyclohexyl, 4-cyclohexyl, 2-furanyl, 2- thienyl, 2-thiazolyl, and 4-hydroxybutyl.

11. The neurotrophic compound of claim 9 having an affinity for FKBP-type immunophilins.

12. The neurotrophic compounds of claim 11, where the FKBP-type immunophilin is FKBP-12.

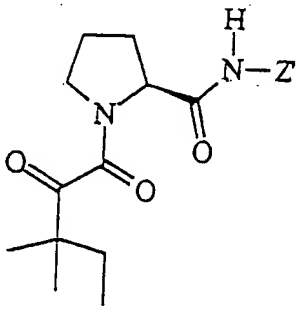
13. The neurotrophic compound of claim 9, capable of inhibiting rotamase activity.

14. The neurotrophic compound of claim 9, where Z and R_1 are liphophilic groups.

15. A pharmaceutical composition comprising a neurotrophically effective amount of the compound of

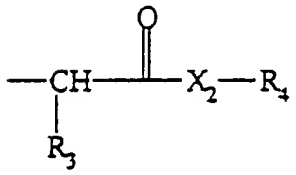
claim 9 and a pharmaceutically acceptable carrier.

16. A neurotrophic compound of the formula:



where

Z' is the fragment:



where

R_3 is selected from the group consisting of straight or branched alkyl C_1-C_8 optionally substituted with C_3-C_8 cycloalkyl, or Ar_1 as defined above, and unsubstituted Ar_1;

X_2 is O or NR_5, where R_5 is selected from the group consisting of hydrogen, C_1-C_6 straight or branched alkyl and alkenyl;

R_4 is selected from the group consisting of phenyl, benzyl, C_1-C_5 straight or branched alkyl or alkenyl, and C_1-C_5 straight or branched alkyl or alkenyl substituted with phenyl; or pharmaceutically acceptable salts or hydrates thereof.

17. The neurotrophic compound of claim 16, having an affinity for FKBP-type immunophilins.

18. The neurotrophic compound of claim 17, where the FKBP-type immunophilin is FKBP-12.

19. The neurotrophic compound of claim 16, capable of inhibiting rotamase activity.

20. The neurotrophic compound of claim 16, where Z' is a lipophilic group.

21. A pharmaceutical composition comprising a neurotrophically effective amount of the compound of claim 16 and a pharmaceutically acceptable carrier.

22. A neurotrophic compound having an affinity for FKBP-type immunophilins wherein the immunophilin exhibits rotamase activity and the neurotrophic compound inhibits the rotamase activity of the immunophilin.

23. The neurotrophic compound of claim 22, wherein the FKBP-type immunophilin is FKBP-12.

24. A method of treating a neurological disorder in an animal comprising administering a therapeutically effective amount of a compound having an affinity for FKBP-type immunophilins wherein the immunophilin exhibits rotamase activity and the neurotrophic compound inhibits the rotamase activity of the immunophilin.

25. The method of claim 24, wherein the FKBP-type immunophilin is FKBP-12.

26. The method of claim 24, wherein the neurological disorder is selected from the group consisting of peripheral neuropathies, and neurological pathologies related to neurodegeneration.

27. The method of claim 24, wherein the neurological disorder is Alzheimer's disease.

28. The method of claim 24, wherein the neurological disorder is Parkinson's disease.

29. The method of claim 24, wherein the neurological disorder is amyotrophic lateral sclerosis.

30. A method of promoting neuronal regeneration and growth in mammals, comprising administering to a subject an effective amount of a neurotrophic compound having an affinity for FKBP-type immunophilins wherein the immunophilin exhibits rotamase activity and the neurotrophic compound inhibits the rotamase activity of the immunophilin.

31. The method of claim 30, wherein the FKBP-type immunophilin is FKBP-12.

32. A method of preventing neurodegeneration in an animal comprising administering an effective amount of a compound having an affinity for FKBP-type immunophilins wherein the immunophilin exhibits rotamase activity and the neurotrophic compound inhibits the rotamase activity of the immunophilin.

33. The method of claim 32, wherein the FKBP-type immunophilin is FKBP-12.

ABSTRACT OF THE DISCLOSURE

This invention relates to neurotrophic compounds having an affinity for FKBP-type immunophilins, their preparation and use as inhibitors of the enzyme activity associated with immunophilin proteins, and particularly inhibitors of peptidyl-prolyl isomerase or rotamase enzyme activity.

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(Rev. 8-95)

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Applicant(s)

GREGORY S. HAMILTON, CATONSVILLE, MD; JOSEPH P. STEINER, HAMPSTEAD, MD.

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TITLE
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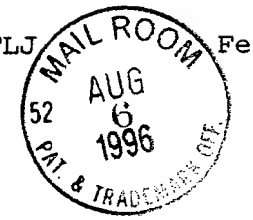
Attorney Docket No. 22762-Y

Date: August 6, 1996 Attorney: GMN/TLJ Fee: $375.00



Inventor(s): Gregory S. Hamilton and
Joseph P. Steiner

Serial No.: Not Yet Assigned

Filed: August 6, 1996

Title: **SMALL MOLECULE INHIBITORS OF ROTAMASE ENZYME ACTIVITY**

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33 claims
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Title:

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BACKGROUND OF THE INVENTION

1. Field of the Invention

This invention relates to neurotrophic compounds having an affinity for FKBP-type immunophilins, their preparation and use as inhibitors of the enzyme activity associated with immunophilin proteins, and particularly inhibitors of peptidyl-prolyl isomerase or rotamase enzyme activity.

2. Description of the Prior Art

The term immunophilin refers to a number of proteins that serve as receptors for the principal immunosuppressant drugs, cyclosporin A (CsA), FK506, and rapamycin. Known classes of immunophilins are cyclophilins, and FK506 binding proteins, such as FKBP. Cyclosporin A binds to cyclophilin while FK506 and rapamycin bind to FKBP. These immunophilin-drug complexes interface with a variety of intracellular signal transduction systems, especially in the immune system and the nervous system.

Immunophilins are known to have peptidyl-prolyl isomerase (PPIase) or rotamase enzyme activity. It has been determined that rotamase activity has a role in the catalyzation of the interconversion of the cis and trans isomer of immunophilin proteins.

Immunophilins were originally discovered and studied in immune tissue. It was initially postulated by those skilled in the art that inhibition of the immunophilins rotamase activity leads to the inhibition

of T-cell proliferation, thereby causing the immunosuppressive action exhibited by immunosuppressive drugs such as cyclosporin A, FK506, and rapamycin. Further study has shown that the inhibition of rotamase activity, in and of itself, is not sufficient for immunosuppressant activity. Schreiber et al., *Science*, 1990 vol. 250 pp. 556-559. It has been shown that the immunophilin-drug complexes interact with ternary protein targets as their mode of action. Schreiber et al., *Cell*, 1991, vol. 66, pp. 807-815. In the case of FKBP-FK506 and FKBP-CsA, the drug-immunophilin complexes bind to the enzyme calcineurin, inhibitory T-cell receptor signalling leading to T-cell proliferation. Similarly, the complex of rapamycin and FKBP interacts with the RAFT1/FRAP protein and inhibits signalling from the IL-2 receptor.

Immunophilins have been found to be present at high concentrations in the central nervous system. Immunophilins are enriched 10-50 times more in the central nervous system than in the immune system. Within neural tissues, immunophilins appear to influence neuronal process extension, nitric oxide synthesis, and neurotransmitter release.

It has been found that picomolar concentrations of an immunosuppressant such as FK506 and rapamycin stimulate neurite out growth in PC12 cells and sensory nervous, namely dorsal root ganglion cells (DRGs). Lyons et al., *Proc. of Natl. Acad. Sci.*, 1994 vol. 91,

pp. 3191-3195. In whole animal experiments, FK506 has been shown to stimulate nerve regeneration following facial nerve injury and results in functional recovery in animals with sciatic nerve lesions.

Surprisingly, it has been found that drugs with a high affinity for FKBP are potent rotamase inhibitors causing a neurotrophic effect. Lyons et al. These findings suggest the use of immunosuppressants in treating various peripheral neuropathies and enhancing neuronal regrowth in the central nervous system (CNS). Studies have demonstrated that neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis (ALS) may occur due to the loss, or decreased availability, of a neurotrophic substance specific for a particular population of neurons affected in the disorder.

Several neurotrophic factors effecting specific neuronal populations in the central nervous system have been identified. For example, it has been hypothesized that Alzheimer's disease results from a decrease or loss of nerve growth factor (NGF). It has thus been proposed to treat Alzheimer's patients with exogenous nerve growth factor or other neurotrophic proteins such as brain derived nerve factor (BDNF), glial derived nerve factor, ciliary neurotrophic factor, and neurotropin-3 to increase the survival of degenerating neuronal populations.

Clinical application of these proteins in various neurological disease states is hampered by difficulties in the delivery and bioavailability of large proteins to nervous system targets. By contrast, immunosuppressant drugs with neurotrophic activity are relatively small and display excellent bioavailability and specificity. However, when administered chronically, immunosuppressants exhibit a number of potentially serious side effects including nephrotoxicity, such as impairment of glomerular filtration and irreversible interstitial fibrosis (Kopp et al., 1991, J. Am. Soc. Nephrol. 1:162); neurological deficits, such as involuntary tremors, or non-specific cerebral angina such as non-localized headaches (De Groen et al., 1987, N. Engl. J. Med. 317:861); and vascular hypertension with complications resulting therefrom (Kahan et al., 1989 N. Engl. J. Med. 321: 1725).

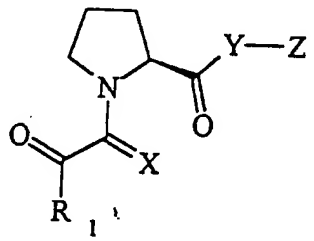
In order to prevent the side effects associated with use of the immunosuppressant compounds, the present invention provides non-immunosuppressive compounds containing small molecule FKBP rotamase inhibitors for promoting neuronal growth and regeneration in various neuropathological situations where neuronal repair can be facilitated including peripheral nerve damage by physical injury or disease state such as diabetes, physical damage to the central nervous system (spinal cord and brain) brain damage

associated with stroke, and for the treatment of neurological disorders relating to neurodegeneration, including Parkinson's disease, Alzheimer's disease, and amyotrophic lateral sclerosis.

SUMMARY OF THE INVENTION

The present invention relates to a novel class of neurotrophic compounds having an affinity for FKBP-type immunophilins. Once bound to this protein the neurotrophic compounds are potent inhibitors of the enzyme activity associated with immunophilin proteins and particularly rotamase enzyme activity, thereby stimulating neuronal regeneration and outgrowth. A key feature of the compounds of the present invention is that they do not exert any significant immunosuppressive activity in addition to their neurotrophic activity.

A preferred embodiment of this invention is a neurotrophic compound of the formula:



where

R_1 is selected from the group consisting of a C_1-C_9 straight or branched chain alkyl or alkenyl group optionally substituted with C_3-C_8 cycloalkyl, C_3 or C_5 cycloalkyl, C_5-C_7 cycloalkenyl, Ar_1, where said alkyl, alkenyl, cycloalkyl or cycloalkenyl groups may be optionally substituted with C_1-C_4 alkyl, C_1-C_4 alkenyl, or hydroxy, where Ar_1 is selected from the group consisting of 1-napthyl, 2-napthyl, 2-indolyl, 3-indolyl, 2-furyl, 3-furyl, 2-thiazolyl, 2-thienyl, 3-thienyl, 2-,3-, 4-pyridyl, and phenyl, having one to three substituents which are independently selected from the group consisting of hydrogen, halo, hydroxyl, nitro, trifluoromethyl, C_1-C_6 straight or branched alkyl or alkenyl, C_1-C_4 alkoxy or C_1-C_4 alkenyloxy, phenoxy, benzyloxy, and amino;

X is selected from the group consisting of oxygen, sulfur, methylene (CH_2), or H_2;

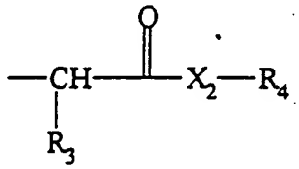
Y is selected from the group consisting of oxygen or NR_2, where R_2 is hydrogen or C_1-C_6 alkyl; and

Z is selected from the group consisting of C_2-C_6 straight or branched chain alkyl or alkenyl,

wherein the alkyl chain is substituted in one or more positions with Ar_1 as defined above, C_3-C_8 cycloalkyl, cycloalkyl connected by a C_1-C_6

straight or unbranched alkyl or alkenyl chain, and Ar_2 where Ar_2 is selected from the group consisting of 2-indolyl, 3-indolyl, 2-furyl, 3-furyl, 2- thiazolyl, 2-thienyl, 3-thienyl, 2-, 3-, or 4-pyridyl, and phenyl, having one to three substituents which are independently selected from the group consisting of hydrogen, halo, hydroxyl, nitro, trifluoromethyl, C_1-C_6 straight or branched alkyl or alkenyl, C_1-C_4 alkoxy or C_1-C_4 alkenyloxy, phenoxy, benzyloxy, and amino;

Z may also be the fragment:



where

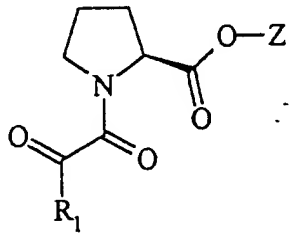
R_3 is selected from the group consisting of straight or branched alkyl C_1-C_8 optionally substituted with C_3-C_8 cycloalkyl, or Ar_1 as defined above, and unsubstituted Ar_1;

X_2 is O or NR_5, where R_5 is selected from the group consisting of hydrogen, C_1-C_6 straight or branched alkyl and alkenyl;

R_4 is selected from the group consisting of phenyl, benzyl, C_1-C_5 straight or branched alkyl or alkenyl, and C_1-C_5 straight or

branched alkyl or alkenyl substituted with phenyl; or pharmaceutically acceptable salts or hydrates thereof.

Another preferred embodiment of this invention is a neurotrophic compound of the formula:



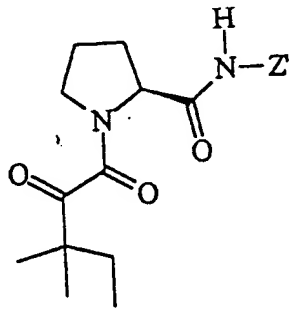
where

R_1 is a C_1-C_9 straight or branched chain alkyl or alkenyl group optionally substituted with C_3-C_8 cycloalkyl, C_3 or C_5 cycloalkyl, C_5-C_7 cycloalkenyl, or Ar_1, where said alkyl, alkenyl, cycloalkyl or cycloalkenyl groups may be optionally substituted with C_1-C_4 alkyl, C_1-C_4 alkenyl, or hydroxy, and where Ar_1 is selected from the group consisting of 1-napthyl, 2-napthyl, 2-indolyl, 3-indolyl, 2-furyl, 3-furyl, 2-thiazolyl, 2-thienyl, 3-thienyl, 2-,3-, or 4-pyridyl, or phenyl, having one to three substituents which are independently selected from the group consisting of hydrogen, halo, hydroxyl, nitro, trifluoromethyl, C_1-C_6 straight or branched alkyl or alkenyl, C_1-C_4 alkoxy or C_1-

C_4 alkenyloxy, phenoxy, benzyloxy, and amino;

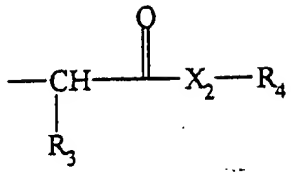
Z is a C_2-C_6 straight or branched chain alkyl or alkenyl, wherein the alkyl chain is substituted in one or more positions with Ar_1 as defined above, C_3-C_8 cycloalkyl, cycloalkyl connected by a C_1-C_6 straight or unbranched alkyl or alkenyl chain, or Ar_2 where Ar_2 is selected from the group consisting of 2-indolyl, 3-indolyl, 2-furyl, 3-furyl, 2- thiazolyl, 2-thienyl, 3-thienyl, 2-, 3-, or 4-pyridyl, or phenyl, having one to three substituents which are independently selected from the group consisting of hydrogen, halo, hydroxyl, nitro, trifluoromethyl, C_1-C_6 straight or branched alkyl or alkenyl, C_1-C_4 alkoxy or C_1-C_4 alkenyloxy, phenoxy, benzyloxy, and amino; or pharmaceutically acceptable salts or hydrates thereof.

Another preferred embodiment of this invention is a neurotrophic compound of the formula:



where

Z' is the fragment:



where

R_3 is selected from the group consisting of straight or branched alkyl C_1-C_8 optionally substituted with C_3-C_8 cycloalkyl, or Ar_1 as defined above, and unsubstituted Ar_1;

X_2 is O or NR_5, where R_5 is selected from the group consisting of hydrogen, C_1-C_6 straight or branched alkyl and alkenyl;

R_4 is selected from the group consisting of phenyl, benzyl, C_1-C_5 straight or branched alkyl or alkenyl, and C_1-C_5 straight or branched alkyl or alkenyl substituted with phenyl; or pharmaceutically acceptable salts or hydrates thereof.

Another preferred embodiment of the invention is a neurotrophic compound having an affinity for FKBP-type immunophilins which inhibit the rotamase activity of the immunophilin.

Another preferred embodiment of the present invention is a method for treating a neurological disorder in an animal comprising administering a therapeutically effective amount of a compound having an affinity for FKBP-type immunophilins which inhibits the rotamase activity of the immunophilin.

Another preferred embodiment of the invention is a method of promoting neuronal regeneration and growth in mammals, comprising administering to a mammal an effective amount of a neurotrophic compound having an affinity for FKBP-type immunophilins which inhibits the rotamase activity of the immunophilin.

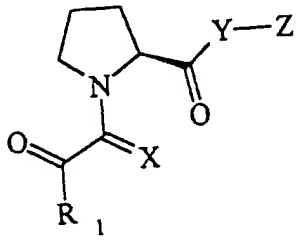
Yet another preferred embodiment of the invention is a method of preventing neurodegeneration in an animal comprising administering to an animal an effective amount of a neurotrophic compound having an affinity for FKBP-type immunophilins which inhibits rotamase activity of the immunophilin.

DETAILED DESCRIPTION OF THE INVENTION

The novel neurotrophic compounds of this invention are relatively small molecules in relation to other known compounds which bind to FKBP-type immunophilins, such as rapamycin, FK506, and cyclosporin.

The neurotrophic compounds of this invention have an affinity for the FK506 binding proteins such as FKBP-12. When the neurotrophic compounds of the invention are bound to the FKBP, they have been found

to unexpectedly inhibit the prolyl- peptidyl cis-trans isomerase activity, or rotamase activity of the binding protein and stimulate neurite growth, while not exhibiting an immunosuppressant effect. More particularly, this invention relates to a novel class of neurotrophic compounds represented by the formula:



I

where

R_1 is a C_1-C_9 straight or branched chain alkyl or alkenyl group optionally substituted with C_3-C_8 cycloalkyl, C_3 or C_5 cycloalkyl, C_5-C_7 cycloalkenyl, or Ar_1, where said alkyl, alkenyl, cycloalkyl or cycloalkenyl groups may be optionally substituted with C_1-C_4 alkyl, C_1-C_4 alkenyl, or hydroxy, and where Ar_1 is selected from the group consisting of 1-napthyl, 2-napthyl, 2-indolyl, 3-indolyl, 2-furyl, 3-furyl, 2- thiazolyl, 2-thienyl, 3-thienyl, 2-, 3-, or 4-pyridyl, or phenyl, having one to three substituents which are independently selected from the group

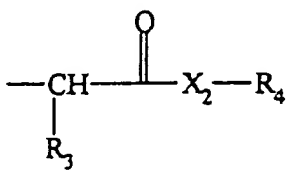
consisting of hydrogen, halo, hydroxyl, nitro, trifluoromethyl, C_1-C_6 straight or branched alkyl or alkenyl, C_1-C_4 alkoxy or C_1-C_4 alkenyloxy, phenoxy, benzyloxy, and amino;

X is oxygen, sulfur, methylene (CH_2), or H_2;

Y is oxygen or NR_2, where R_2 is hydrogen or C_1-C_6 alkyl; and

Z is a C_2-C_6 straight or branched chain alkyl or alkenyl, wherein the alkyl chain is substituted in one or more positions with Ar_1 as defined above, C_3-C_8 cycloalkyl, cycloalkyl connected by a C_1-C_6 straight or unbranched alkyl or alkenyl chain, or Ar_2 where Ar_2 is selected from the group consisting of 2-indolyl, 3-indolyl, 2-furyl, 3-furyl, 2- thiazolyl, 2-thienyl, 3-thienyl, 2-, 3-, or 4-pyridyl, or phenyl, having one to three substituents which are independently selected from the group consisting of hydrogen, halo, hydroxyl, nitro, trifluoromethyl, C_1-C_6 straight or branched alkyl or alkenyl, C_1-C_4 alkoxy or C1-C4 alkenyloxy, phenoxy, benzyloxy, and amino;

Z may also be the fragment:



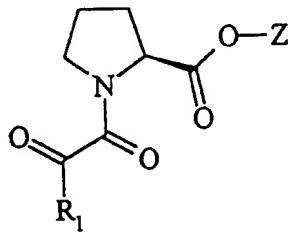
where

R_3 is selected from the group consisting of straight or branched alkyl C_1-C_8 optionally substituted with C_3-C_8 cycloalkyl, or Ar_1 as defined above, and unsubstituted Ar_1;

X_2 is O or NR_5, where R_5 is selected from the group consisting of hydrogen, C_1-C_6 straight or branched alkyl and alkenyl;

R_4 is selected from the group consisting of phenyl, benzyl, C_1-C_5 straight or branched alkyl or alkenyl, and C_1-C_5 straight or branched alkyl or alkenyl substituted with phenyl; or pharmaceutically acceptable salts or hydrates thereof.

Preferred compounds have the following formula:



II

where

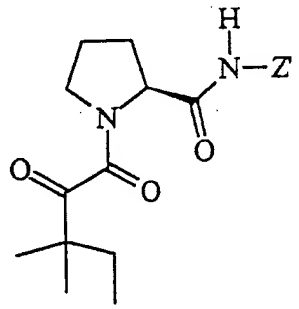
R_1 is a C_1-C_9 straight or branched chain alkyl or alkenyl group optionally substituted with C_3-C_8 cycloalkyl, C_3 or C_5 cycloalkyl, C_5-C_7

cycloalkenyl, or Ar_1, where said alkyl, alkenyl, cycloalkyl or cycloalkenyl groups may be optionally substituted with C_1-C_4 alkyl, C_1-C_4 alkenyl, or hydroxy, and where Ar_1 is selected from the group consisting of 1-napthyl, 2-napthyl, 2-indolyl, 3-indolyl, 2-furyl, 3-furyl, 2-thiazolyl, 2-thienyl, 3-thienyl, 2-, 3-, or 4-pyridyl, or phenyl, having one to three substituents which are independently selected from the group consisting of hydrogen, halo, hydroxyl, nitro, trifluoromethyl, C_1-C_6 straight or branched alkyl or alkenyl, C_1-C_4 alkoxy or C_1-C_4 alkenyloxy, phenoxy, benzyloxy, and amino;

Z is a C_2-C_6 straight or branched chain alkyl or alkenyl, wherein the alkyl chain is substituted in one or more positions with Ar_1 as defined above, C3-C8 cycloalkyl, cycloalkyl connected by a C_1-C_6 straight or unbranched alkyl or alkenyl chain, or Ar_2 where Ar_2 is selected from the group consisting of 2-indolyl, 3-indolyl, 2-furyl, 3-furyl, 2- thiazolyl, 2-thienyl, 3-thienyl, 2-, 3-, or 4-pyridyl, or phenyl, having one to three substituents which are independently selected from the group consisting of hydrogen, halo, hydroxyl, nitro, trifluoromethyl, C_1-C_6 straight or

branched alkyl or alkenyl, C_1-C_4 alkoxy or C_1-C_4 alkenyloxy, phenoxy, benzyloxy, and amino; or pharmaceutically acceptable salts or hydrates thereof.

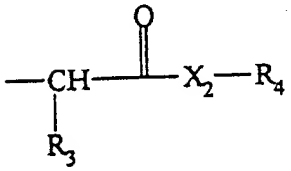
In another preferred embodiment novel compounds of this invention are represented by the formula:



III

where

Z' is the fragment:



where

R_3 is selected from the group consisting of straight or branched alkyl C_1-C_8 optionally substituted with C_3-C_8 cycloalkyl, or Ar_1 as defined above, or unsubstituted Ar_1;

X_2 is O or NR_5, where R_5 is selected from the group consisting of hydrogen, C_1-C_6 straight or branched alkyl and alkenyl;

R_4 is selected from the group consisting of phenyl, benzyl, C_1-C_5 straight or branched alkyl or alkenyl, and C_1-C_5 straight or branched alkyl or alkenyl substituted with phenyl; or pharmaceutically acceptable salts or hydrates thereof.

The compounds of this invention exist as stereoisomeric forms, either enantiomers or diastereoisomers. The stereochemistry at position 1 (Formula 1) is R or S, with S preferred. Included within the scope of the invention are the enantiomers, the racemic form, and diastereoisomeric mixtures. Enantiomers as well as diastereoisomers can be separated by methods known to those skilled in the art.

It is known that immunophilins such as FKBP preferentially recognize peptide substrates containing Xaa-Pro-Yaa motifs, where Xaa and Yaa are lipophilic amino acid residues. Schreiber et al. 1990 *J. Org. Chem.* 55, 4984-4986; Harrison and Stein, 1990 *Biochemistry*, 29, 3813-3816. Thus modified prolyl peptidomimetic compounds bearing lipophilic substituents should bind with high affinity to the hydrophobic core of the FKBP active site and inhibit its rotamase activity.

Preferred compounds of the invention include:

3-phenyl-1-propyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate,

3-phenyl-1-prop-2-(E)-enyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate,

3-(3,4,5-trimethoxyphenyl)-1-propyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate,

3-(3,4,5-trimethoxyphenyl)-1-prop-2-(E)-enyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate,

3-(4,5-dichlorophenyl)-1-propyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate,

3-(4,5-dichlorophenyl)-1-prop-2-(E)-enyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate,

3-(4,5-methylenedioxyphenyl)-1-propyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate,

3-(4,5-methylenedioxyphenyl)-1-prop-2-(E)-enyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate,

3-cyclohexyl-1-propyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate,

3-cyclohexyl-1-prop-2-(E)-enyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate,

(1R)-1,3-diphenyl-1-propyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate,

(1R)-1,3-diphenyl-1-prop-2-(E)-enyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate,

(1R)-1-cyclohexyl-3-phenyl-1-propyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate,

(1R)-1-cyclohexyl-3-phenyl-1-prop-2-(E)-enyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate,

(1R)-1-(4,5-dichlorophenyl)-3-phenyl-1-propyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate,

3-phenyl-1-propyl (2S)-1-(1,2-dioxo-2-cyclohexyl)ethyl-2-pyrrolidinecarboxylate,

3-phenyl-1-propyl (2S)-1-(1,2-dioxo-4-cyclohexyl)butyl-2-pyrrolidinecarboxylate,

3-phenyl-1-propyl (2S)-1-(1,2-dioxo-2-[2-furanyl])ethyl-2-pyrrolidinecarboxylate,

3-phenyl-1-propyl (2S)-1-(1,2-dioxo-2-[2-thienyl])ethyl-2-pyrrolidinecarboxylate,

3-phenyl-1-propyl (2S)-1-(1,2-dioxo-2-[2-thiazolyl])ethyl-2-pyrrolidinecarboxylate,

3-phenyl-1-propyl (2S)-1-(1,2-dioxo-2-phenyl)ethyl-2-pyrrolidinecarboxylate,

1,7-diphenyl-4-heptyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate,

3-Phenyl-1-propyl (2S)-1-(3,3-dimethyl-1,2-dioxo-4-hydroxybutyl)-2-pyrrolidinecarboxylate,

3-phenyl-1-propyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxamide,

1-[1-(3,3-dimethyl-1,2-dioxopentyl)-L-proline]-L-phenylalanine ethyl ester,

1-[1-(3,3-dimethyl-1,2-dioxopentyl)-L-proline]-L-leucine ethyl ester,

1-[1-(3,3-dimethyl-1,2-dioxopentyl)-L-proline]-L-phenylglycine ethyl ester,

1-[1-(3,3-dimethyl-1,2-dioxopentyl)-L-proline]-L-phenylalanine phenyl ester,

1-[1-(3,3-dimethyl-1,2-dioxopentyl)-L-proline]-L-phenylalanine benzyl ester, and

1-[1-(3,3-dimethyl-1,2-dioxopentyl)-L-proline]-L-isoleucine ethyl ester.

The compounds of the present invention can be used in the form of salts derived from inorganic or organic acids and bases. Included among such acid salts are the following: acetate, adipate, alginate, aspartate, benzoate, benzenesulfonate, bisulfate butyrate, citrate, camphorate, camphorsulfonate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, fumarate, glucoheptanoate, glycerophosphate, hemissulfate heptanoate, hexanoate, hydrochloride, hydrobromide, hydroiodide, 2-hydroxyethanesulfonate, lactate, maleate, methanesulfonate, 2-naphthalensulfonate, nicotinate, oxalate, pamoate, pectinate, propionate, succinate, tartrate, thiocyanate, tosylate and undecanoate. Base salts include ammonium salts, alkali metal salts such as sodium and potassium salts, alkaline earth metal salts such as calcium and magnesium salts, salt with organic bases such as dicyclohexylamine salts, N-methyl-D-glucamine, and salts with amino acids such as arginine, lysine, and so forth. Also, the basic

nitrogen-containing groups can be quarternized with such agents as lower alkyl halides, such as methyl, ethyl, propyl, and butyl chloride, bromides and iodides; dialkyl sulfates like dimethyl, diethyl, dibutyl and diamyl sulfates, long chain halides such as decyl, lauryl, myristyl and stearyl chlorides, bromides and iodides, aralkyl halides like benzyl and phenethyl bromides and others. Water or oil-soluble or dispersible products are thereby obtained.

The neurotrophic compounds of this invention can be periodically administered to a patient undergoing treatment for neurological disorders or for other reasons in which it is desirable to stimulate neuronal regeneration and growth, such as in various peripheral neuropathic and neurological disorders relating to neurodegeneration. The compounds of this invention can also be administered to mammals other than humans for treatment of various mammalian neurological disorders.

The novel compounds of the present invention are potent inhibitors of rotamase activity and possess an excellent degree of neurotrophic activity. This activity is useful in the stimulation of damaged neurons, the promotion of neuronal regeneration, the prevention of neurodegeneration, and in the treatment of several neurological disorders known to be associated with neuronal degeneration and peripheral neuropathies. The neurological disorders that may be treated include but are not limited to: trigeminal

neuralgia, glossopharyngeal neuralgia, Bell's Palsy, myasthenia gravis, muscular dystrophy, amyotrophic lateral sclerosis, progressive muscular atrophy, progressive bulbar inherited muscular atrophy, herniated, ruptured or prolapsed invertabrae disk syndromes, cervical spondylosis, plexus disorders, thoracic outlet destruction syndromes, peripheral neuropathic such as those caused by lead, dapsone, ticks, prophyria, or Gullain-Barré syndrome, Alzheimer's disease, and Parkinson's disease.

For these purposes the compounds of the present invention may be administered orally, parenterally, by inhalation spray, topically, rectally, nasally, buccally, vaginally or via an implanted reservoir in dosage formulations containing conventional non-toxic pharmaceutically-acceptable carriers, adjuvants and vehicles. The term parenteral as used herein includes subcutaneous, intravenous, intramuscular, intraperitoneally, intrathecally, intraventricularly, intrasternal and intracranial injection or infusion techniques.

To be effective therapeutically as central nervous system targets the immunophilin-drug complex should readily penetrate the blood-brain barrier when peripherally administered. Compounds of this invention which cannot penetrate the blood-brain barrier can be effetively administered by an intraventricular route.

The pharmaceutical compositions may be in the form of a sterile injectable preparation, for example as a sterile injectable aqueous or oleaginous suspension. This suspension may be formulated according to techniques know in the art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent, for example as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono- or diglycerides. Fatty acids such as oleic acid and its glyceride derivatives find use in the preparation of injectables, olive oil or castor oil, especially in their polyoxyethylated versions. These oil solutions or suspensions may also contain a long-chain alcohol diluent or dispersant.

The compounds may be administered orally in the form of capsules or tablets, for example, or as an aqueous suspension or solution. In the case of tablets for oral use, carriers which are commonly used include lactose and corn starch. Lubricating agents, such as magnesium stearate, are also typically added. For oral

administration in a capsule form, useful diluents include lactose and dried corn starch. When aqueous suspensions are required for oral use, the active ingredient is combined with emulsifying and suspending agents. If desired, certain sweetening and/or flavoring and/or coloring agents may be added.

The compounds of this invention may also be administered in the form of suppositories for rectal administration of the drug. These compositions can be prepared by mixing the drug with a suitable non-irritating excipient which is solid at room temperature but liquid at rectal temperature and therefore will melt in the rectum to release the drug. Such materials include cocoa butter, beeswax and polyethylene glycols.

The compounds of this invention may also be administered optically, especially when the conditions addressed for treatment involve areas or organs readily accessible by topical application, including neurological disorders of the eye, the skin, or the lower intestinal tract. Suitable topical formulations are readily prepared for each of these areas.

For ophthalmic use, the compounds can be formulated as micronized suspensions in isotonic, pH adjusted sterile saline, or, preferably, as solutions is isotonic, pH adjusted sterile saline, either with or without a preservative such as benzylalkonium chloride. Alternatively for the ophthalmic uses the compounds may be formulated in an ointment such as petrolatum.

For application topically to the skin, the compounds can be formulated in a suitable ointment containing the compound suspended or dissolved in, for example, a mixture with one or more of the following: mineral oil, liquid petrolatum, white petrolatum, propylene glycol, polyoxyethylene polyoxypropylene compound, emulsifying wax and water. Alternatively, the compounds can be formulated in a suitable lotion or cream containing the active compound suspended or dissolved in, for example, a mixture of one or more of the following: mineral oil, sorbitan monostearate, polysorbate 60, cetyl esters wax, cetearyl alcohol, 2-octyldodecanol, benzyl alcohol and water.

Topical application for the lower intestinal tract an be effected in a rectal suppository formulation (see above) or in a suitable enema formulation.

Dosage levels on the order of about .1mg to about 10,000 mg. of the active ingredient compound are useful in the treatment of the above conditions, with preferred levels of about 0.1mg to about 1,000 mg. The amount of active ingredient that may be combined with the carrier materials to produce a single dosage form will vary depending upon the host treated and the particular mode of administration.

It is understood, however, that a specific dose level for any particular patient will depend upon a variety of factors including the activity of the specific compound employed, the age, body weight,

general health, sex, diet, time of administration, rate of excretion, drug combination, and the severity of the particular disease being treated and form of administration.

The compounds can be administered with other neurotrophic agents such as neurotrophic growth factor (NGF), glial derived growth factor, brain derived growth factor, ciliary neurotrophic factor, and neurotropin-3. The dosage level of other neurotrophic drugs will depend upon the factors previously stated and the neurotrophic effectiveness of the drug combination.

K_i Test Procedure

Inhibition of the peptidyl-prolyl isomerase (rotamase) activity of the inventive compounds can be evaluated by known methods described in the literature (Harding, M.W. et al. *Nature* 341: 758-760 (1989); Holt et al. *J. Am. Chem. Soc.* 115: 9923-9938). These values are obtained as apparent K_i's and are presented for some of Examples 1-30 in Table I. The *cis-trans* isomerization of an alanine-proline bond in a model substrate, N-succinyl-Ala-Ala-Pro-Phe-*p*-nitroanilide, is monitored spectrophotometrically in a chymotrypsin-coupled assay, which releases *para*-nitroanilide from the *trans* form of the substrate. The inhibition of this reaction caused by the addition of different concentrations of inhibitor is determined, and the data is analyzed as a change in first-order rate constant as

a function of inhibitor concentration to yield the apparent K_i values.

In a plastic cuvette are added 950 mL of ice cold assay buffer (25 mM HEPES, pH 7.8, 100 mM NaCl), 10 mL of FKBP (2.5 mM in 10 mM Tris-Cl pH 7.5, 100 mM NaCl, 1 mM dithiothreitol), 25 mL of chymotrypsin (50 mg/ml in 1 mM HCl) and 10 mL of test compound at various concentrations in dimethyl sulfoxide. The reaction is initiated by the addition of 5 mL of substrate (succinyl-Ala-Phe-Pro-Phe-para-nitroanilide, 5 mg/mL in 2.35 mM LiCl in trifluoroethanol).

The absorbance at 390 nm versus time is monitored for 90 sec using a spectrophotometer and the rate constants are determined from the absorbance versus time data files.

The data for these experiments is presented in Table I.

TABLE I

FKBP ROTAMASE INHIBITION

Example	K_inM
4	42
5	125
6	200
7	65
8	2500
9	160
10	52
24	9000

In mammalian cells, FKBP-12 complexes with the inositol triphosphate receptor (IP_3R) and the ryanodine receptor (RyR). It is believed that the neurotrophic compounds of this invention disassociates FKBP-12 from these complexes causing the calcium channel to become "leaky" (Cameron et al., 1995). Calcium fluxes are involved in neurite extensions so that the IP_3R receptor and the ryanodine receptor might be involved in the neurotrophic effects of drugs. Since the drugs bind to the same site as FKBP-12 as the IP_3R receptor, one could assume that the drugs displace the channels from FKBP-12.

Chick Dorsal Root Ganglion Cultures and Neurite Outgrowth

Dorsal root ganglia were dissected from chick embryos of ten day gestation. Whole ganglion explants were cultured on thin layer Matrigel-coated 12 well plates with Liebovitz L15 plus high glucose media supplemented with 2mM glutamine and 10% fetal calf serum, and also containing 10 μM cytosine β-D arabinofuranoside (Ara C) at 37°C in an environment containing 5% CO_2. Twenty-four hours later, the DRGs were treated with various concentrations of nerve growth factor, immunophilin ligands or combinations of NFG plus drugs. Forty-eight hours after drug treatment, the ganglia were visualized under phase contrast or Hoffman Modulation contrast with a Zeiss Axiovert inverted microscope. Photomicrographs of the explants were made, and neurite outgrowth was quantitated. Neurites longer than the DRG diameter were counted as positive, with total number of neurites quantitated per each experimental condition. Three to four DRGs are cultured per well, and each treatment was performed in duplicate.

The data for these experiments are presented in Table II.

TABLE II

Neurite Outgrowth in Chick DRG

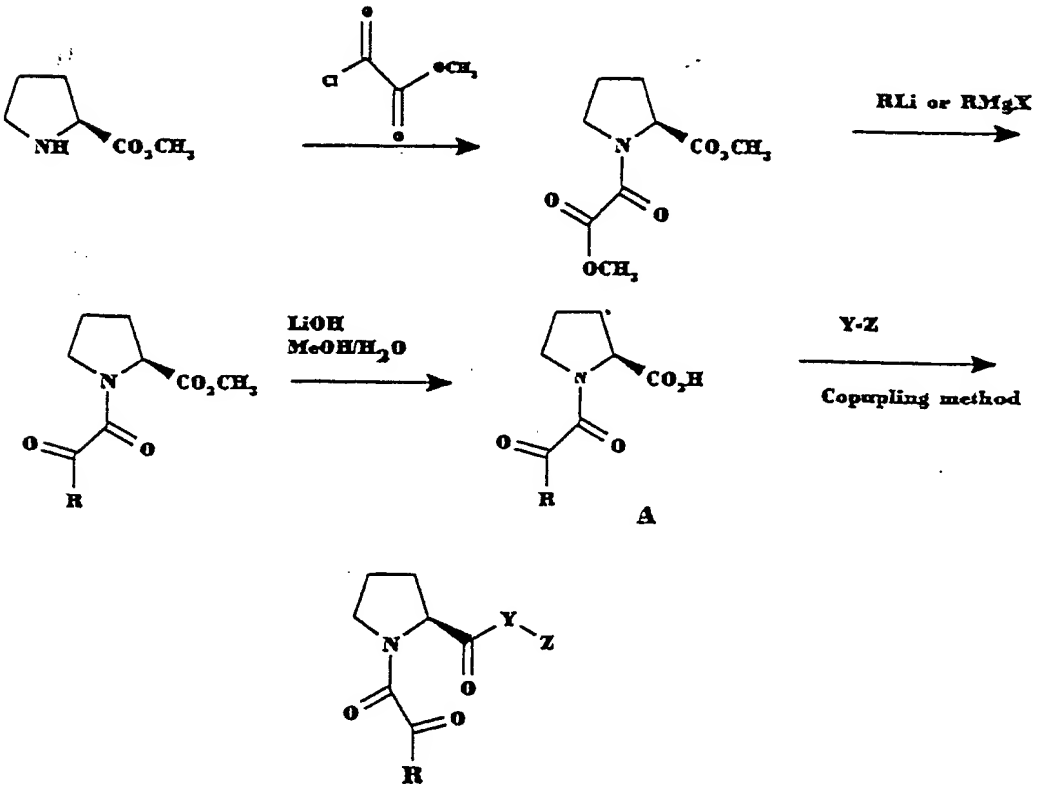
Example	ED_{50} (nM)
4	53
5	105
6	149
7	190
8	850
9	75
10	------
24	------

The following examples are illustrative of preferred embodiments of the invention and are not to be construed as limiting the invention thereto. All polymer molecular weights are mean average molecular weights. All percentages are based on the percent by weight of the final delivery system or formulation prepared unless otherwise indicated and all totals equal 100% by weight.

EXAMPLES

The inventive compounds may be prepared by a variety of synthetic sequences that utilize established chemical transformations. The general pathway to the present compounds is described in Scheme 1. N-glyoxylproline derivatives may be prepared by reacting

L-proline methyl ester with methyl oxalyl chloride as shown in Scheme I. The resulting oxamates may be reacted with a variety of carbon nucleophiles to obtain intermediates compounds. These intermediates are then reacted with a variety of alcohols, amides, or protected amino acid residues to obtain the propyl esters and amides of the invention.



Scheme I

EXAMPLE 1

Synthesis of methyl (2S)-1-(1,2-dioxo-2-methoxyethyl)-2-pyrrolidinecarboxylate.

A solution of L-proline methyl ester hydrochloride (3.08 g; 18.60 mmol) in dry methylene chloride was cooled to 0°C and treated with triethylamine (3.92 g; 38.74 mmol; 2.1 eq). After stirring the formed slurry under a nitrogen atmosphere for 15 min, a solution of methyl oxalyl chloride (3.20 g; 26.12 mmol) in methylene chloride (45 mL) was added dropwise. The resulting mixture was stirred at 0°C for 1.5 hr. After filtering to remove solids, the organic phase was washed with water, dried over $MgSO_4$ and concentrated. The crude residue was purified on a silica gel column, eluting with 50% ethyl acetate in hexane, to obtain 3.52 g (88%) of the product as a reddish oil. Mixture of cis-trans amide rotamers; data for trans rotamer given. ^1H NMR ($CDCl_3$): d 1.93 (dm, 2H); 2.17 (m, 2H); 3.62 (m, 2H); 3.71 (s, 3H); 3.79, 3.84 (s, 3H total); 4.86 (dd, 1H, J = 8.4, 3.3).

EXAMPLE 2

General procedure for the synthesis of pyrrolidinyl alkyl oxamates. Exemplified for methyl (2S)-1-(1,2-dioxo-3,3-dimethylpentyl)-2-pyrrolidinecarboxylate.

A solution of methyl (2S)-1-(1,2-dioxo-2-methoxyethyl)-2-pyrrolidinecarboxylate (2.35 g; 10.90

mmol) in 30 mL of tetrahydrofuran (THF) was cooled to -78°C and treated with 14.2 mL of a 1.0 M solution of 1,1-dimethylpropylmagnesium chloride in THF. After stirring the resulting homogeneous mixture at -78°C for three hours, the mixture was poured into saturated ammonium chloride (100 mL) and extracted into ethyl acetate. The organic phase was washed with water, dried, and concentrated, and the crude material obtained upon removal of the solvent was purified on a silica gel column, eluting with 25% ethyl acetate in hexane, to obtain 2.10 g (75%) of the oxamate as a colorless oil. ^{1}H NMR ($CDCl_3$): d 0.88 (t, 3H); 1.22, 1.26 (s, 3H each); 1.75 (dm, 2H); 1.87-2.10 (m, 3H); 2.23 (m, 1H); 3.54 (m, 2H); 3.75 (s, 3H); 4.52 (dm, 1H, J = 8.4, 3.4).

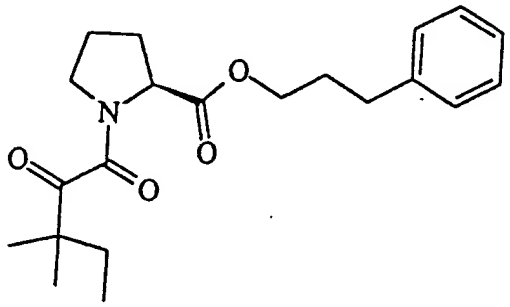
EXAMPLE 3

General procedure for the preparation of pyrrolidine carboxylic acids. Exemplified for (2S)-1-(1,2-dioxo-3,3-dimethylpentyl)-2-pyrrolidinecarboxylic acid.

A mixture of methyl (2S)-1-(1,2-dioxo-3,3-dimethylpentyl)-2-pyrrolidinecarboxylate (2.10 g; 8.23 mmol), 1 N LiOH (15 mL), and methanol (50 mL) was stirred at 0°C for 30 min and at room temperature overnight. The mixture was acidified to pH 1 with 1 N HCl, diluted with water, and extracted into 100 mL of methylene chloride. The organic extract was washed with

brine and concentrated to deliver 1.73 g (87%) of snow-white solid which did not require further purification. ^{1}H NMR ($CDCl_3$): d 0.87 (t, 3H); 1.22, 1.25 (s, 3H each); 1.77 (dm, 2H); 2.02 (m, 2H); 2.17 (m, 1H); 2.25 (m, 1H); 3.53 (dd, 2H, J = 10.4, 7.3); 4.55 (dd, 1H, J = 8.6, 4.1).

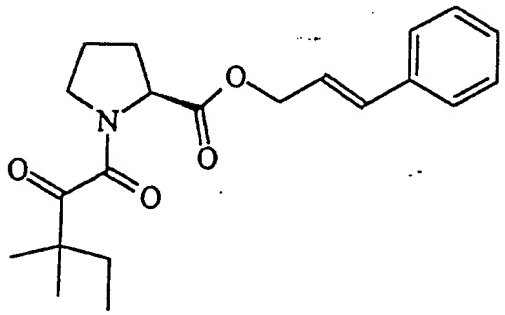
EXAMPLE 4



General procedure for the synthesis of prolyl esters. Exemplified for 3-phenyl-1-propyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate. A mixture of (2S)-1-(1,2-dioxo-3,3-dimethylpentyl)-2-pyrrolidine-carboxylic acid (600 mg; 2.49 mmol), 3-phenyl-1-propanol (508 mg; 3.73 mmol), dicyclohexylcarbodiimide (822 mg; 3.98 mmol), camphorsulphonic acid (190 mg; 0.8 mmol) and 4-dimethylaminopyridine (100 mg; 0.8 mmol) in methylene chloride (20 mL) was stirred overnight under a nitrogen atmosphere. The reaction mixture was filtered through Celite to remove solids and concentrated in vacuo, and the crude material was purified on a flash column (25% ethyl acetate in hexane) to obtain 720 mg (80%) of the

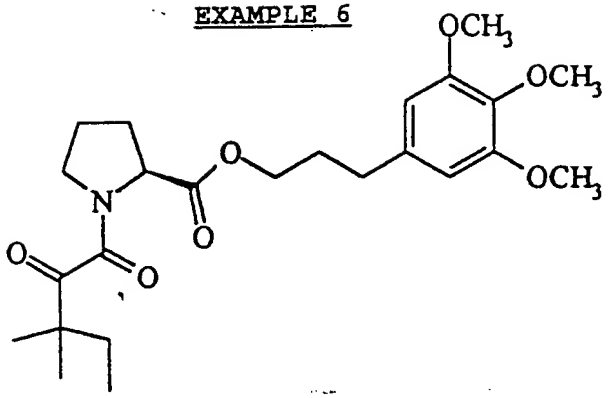
product as a colorless oil. ^1H NMR ($CDCl_3$): d 0.84 (t, 3H); 1.19 (s, 3H); 1.23 (s, 3H); 1.70 (dm, 2H); 1.98 (m, 5H); 2.22 (m, 1H); 2.64 (m, 2H); 3.47 (m, 2H); 4.14 (m, 2H); 4.51 (d, 1H); 7.16 (m, 3H); 7.26 (m, 2H).

EXAMPLE 5



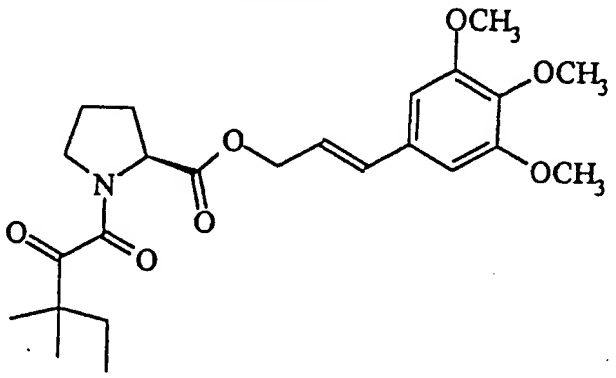
3-Phenyl-1-prop-2-(E)-enyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate, 80%, ^1H NMR (360 Mhz, $CDCl_3$): d 0.86 (t, 3H); 1.21 (s, 3H); 1.25 (s, 3H); 1.54-2.10 (m, 5H); 2.10-2.37 (m, 1H); 3.52-3.55 (m, 2H); 4.56 (dd, 1H, J = 3.8, 8.9); 4.78-4.83 (m, 2H); 6.27 (m, 1H); 6.67 (dd, 1H, J = 15.9); 7.13-7.50 (m, 5H). This compound was prepared by the method of Example 3 from (2S)-1-(1,2-dioxo-3,3-dimethylpentyl)-2-pyrrolidine-carboxylic acid.

EXAMPLE 6



3-(3,4,5-Trimethoxyphenyl)-1-propyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate, 61%, ^{1}H NMR ($CDCl_3$): d 0.84 (t, 3H); 1.15 (s, 3H); 1.24 (s, 3H); 1.71 (dm, 2H); 1.98 (m, 5H); 2.24 (m, 1H); 2.63 (m, 2H); 3.51 (t, 2H); 3.79 (s, 3H); 3.83 (s, 3H); 4.14 (m, 2H); 4.52 (m, 1H); 6.36 (s, 2H). This compound was prepared by the method of Example 3 from (2S)-1-(1,2-dioxo-3,3-dimethylpentyl)-2-pyrrolidine-carboxylic acid.

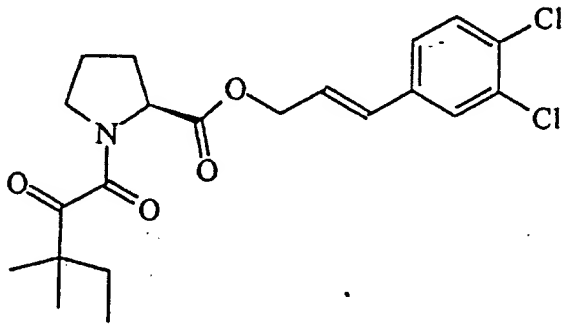
EXAMPLE 7



3-(3,4,5-Trimethoxyphenyl)-1-prop-2-(E)-enyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate, 66%, ^{1}H NMR ($CDCl_3$): d 0.85 (t,

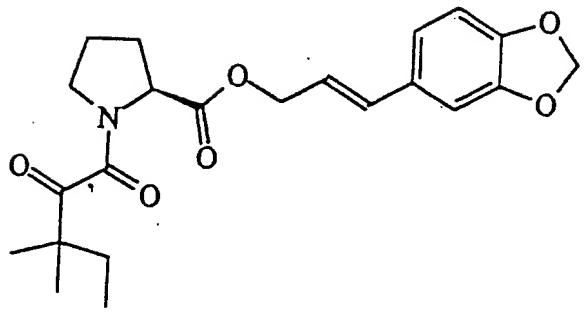
3H); 1.22 (s, 3H); 1.25 (s, 3H); 1.50-2.11 (m, 5H); 2.11-2.40 (m, 1H); 3.55 (m, 2H); 3.85 (s, 3H); 3.88 (s, 6H); 4.56 (dd, 1H); 4.81 (m, 2H); 6.22 (m, 1H); 6.58 (d, 1H, J = 16); 6.63 (s, 2H). This compound was prepared by the method of Example 3 from (2S)-1-(1,2-dioxo-3,3-dimethylpentyl)-2-pyrrolidine-carboxylic acid.

EXAMPLE 8



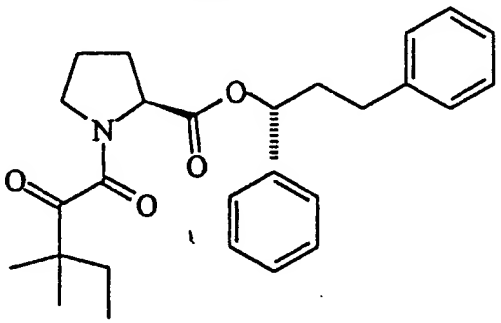
3-,4,5-Dichlorophenyl)-1-prop-2-(E)-enyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate, 70%, ^{1}H NMR ($CDCl_3$): d 0.85 (t, 3H); 1.21 (s, 3H); 1.25 (s, 3H); 1.51-1.87 (m, 2H); 1.87-2.39 (m, 4H); 3.51-3.57 (m, 2H); 4.50-4.61 (dd, 1H, J = 3.4, 8.6); 4.80 (d, 2H, J = 6.0); 6.20-6.34 (m, 1H); 6.50-6.66 (d, 1H, J = 16); 7.13-7.24 (dd, 1H, J = 1.8, 8.3); 7.39 (d, 1H, J = 8.3); 7.47 (s, 1H). This compound was prepared by the method of Example 3 from (2S)-1-(1,2-dioxo-3,3-dimethylpentyl)-2-pyrrolidine-carboxylic acid.

EXAMPLE 9



3-(4,5-Methylenedioxyphenyl)-1-prop-2-(E)-enyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate, 82%, ^{1}H NMR (360 MHz, $CDCl_3$): d 0.86 (t,3H); 1.22 (s, 3H); 1.25 (s, 3H); 1.60-2.10 (m, 5H); 3.36-3.79 (m, 2H); 4.53 (dd, 1H, J = 3.8, 8.6); 4.61-4.89 (m, 2H); 5.96 (s, 2H); 6.10 (m, 1H); 6.57 (dd, 1H, J = 6.2, 15.8); 6.75 (d, 1H, J = 8.0); 6.83 (dd, 1H, J = 1.3, 8.0); 6.93 (s, 1H). This compound was prepared by the method of Example 3 from (2S)-1-(1,2-dioxo-3,3-dimethylpentyl)-2-pyrrolidine-carboxylic acid.

EXAMPLE 10

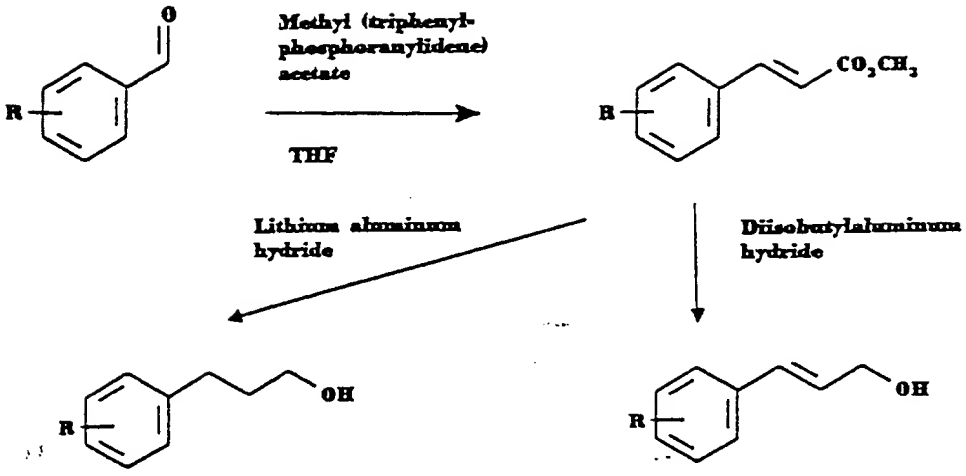


(1R)-1,3-Diphenyl-1-propyl (2S)-1-(3,3-dimethyl-

1,2-dioxopentyl)-2-pyrrolidinecarboxylate, 90%, ^{1}H NMR (360 MHz, $CDCl_3$): d 0.85 (t, 3H); 1.20 (s, 3H); 1.23 (s, 3H); 1.49-2.39 (m, 7H); 2.46-2.86 (m, 2H); 3.25-3.80 (m, 2H); 4.42-4.82 (m, 1H); 5.82 (td, 1H, J = 1.8, 6.7); 7.05-7.21 (m, 3H); 7.21-7.46 (m, 7H). This compound was prepared by the method of Example 3 from (2S)-1-(1,2-dioxo-3,3-dimethylpentyl)-2-pyrrolidine-carboxylic acid.

EXAMPLE 11

The requisite substituted alcohols may be prepared by a number of methods known to those skilled in the art of organic synthesis. As described in Scheme II, substituted benzaldehydes may be homologated to phenyl propanols by reaction with methyl (triphenylphosphoranylidene)- acetate to provide a variety of *trans*-cinnamates; these latter may be reduced to the saturated alcohols by reaction with excess lithium aluminum hydride, or sequentially by reduction of the double bond by catalytic hydrogenation and reduction of the saturated ester by appropriate reducing agents. Alternatively, the *trans*-cinnamates may be reduced to (E)-allylic alcohols by the use of diisobutylaluminum hydride.



Scheme II

Longer chain alcohols may be prepared by homologation of benzylic and higher aldehydes. Alternatively, these aldehydes may be prepared by conversion of the corresponding phenylacetic and higher acids, and phenethyl and higher alcohols.

EXAMPLE 12

General procedure for the synthesis of acrylic esters, exemplified for methyl (3,3,5-trimethoxy)-*trans*-cinnamate.

A solution of 3,4,5-trimethoxybenzaldehyde (5.0 g; 25.48 mmol) and methyl (triphenyl-phosphoranylidene)acetate (10.0 g; 29.91 mmol) in tetrahydrofuran (250 mL) was refluxed overnight. After cooling, the reaction mixture was diluted with 200 mL of ethyl acetate and washed with 2 x 200 mL of water, dried, and concentrated in vacuo. The crude residue was chromatographed on a silica gel column, eluting with

25% ethyl acetate in hexane, to obtain 5.63 g (88%) of the cinnamate as a white crystalline solid, ^{1}H NMR (300 Mhz; $CDCl_3$): d 3.78 (s, 3H); 3.85 (s, 6H); 6.32 (d, 1H, J = 16); 6.72 (s, 2H); 7.59 (d, 1H, J = 16).

EXAMPLE 13

Methyl (4,5-dichloro)-*trans*-cinnamate, 80%, ^{1}H NMR (300 Mhz; $CDCl_3$): d 3.79 (s, 3H); 6.40 (d, 1H, J = 16.8); 7.32 (dd, 1H, J = 1.5, 8.1); 7.44 (d, 1H, J = 8.1); 7.56 (d, 1H, J = 16); 7.58 (s, 1H). This compound was prepared by the method of Example 11 from 3,4,5-trimethoxybenzaldehyde.

EXAMPLE 14

Methyl (4,5-methylenedioxy)-*trans*-cinnamate, 74%, ^{1}H NMR (360 Mhz; $CDCl_3$): d 3.79 (s, 3H); 6.01 (s, 2H); 6.26 (d, 1H, J = 16); 6.81 (d, 1H, J = 7.9); 7.00 (d, 1H, J = 8.2); 7.03 (s, 1H); 7.60 (d, 1H, J = 16). This compound was prepared by the method of Example 11 from 3,4,5-trimethoxybenzaldehyde.

EXAMPLE 15

Methyl (2-cyclohexyl)-(E)-acrylate, 80%, ^{1}H NMR (360 Mhz; $CDCl_3$): d 1.12-1.43 (m, 5H); 1.52-1.87 (m, 5H); 2.12 (m, 1H); 3.71 (s, 3H); 5.77 (dd, 1H, J = 1.2, 15.8); 6.92 (dd, 1H, J = 6.8, 15.8). This compound was prepared by the method of Example 11 from 3,4,5-trimethoxybenzaldehyde.

EXAMPLE 16

General procedure for the synthesis of saturated alcohols from acrylic esters. Exemplified for (3,4,5-

trimethoxy) phenylpropanol.

A solution of methyl (3,3,5-trimethoxy)-*trans*-cinnamate (1.81 g; 7.17 mmol) in tetrahydrofuran (30 mL) was added in a dropwise manner to a solution of lithium aluminum hydride (14 mmol) in THF (35 mL), with stirring and under an argon atmosphere. After the addition was complete, the mixture was heated to 75°C for 4 hours. After cooling, it was quenched by the careful addition of 15 mL of 2N NaOH followed by 50 mL of water. The resulting mixture was filtered through Celite to remove solids, and the filter cake was washed with ethyl acetate. The combined organic fractions were washed with water, dried, concentrated in vacuo, and purified on a silica gel column, eluting with ethyl acetate to obtain 0.86 g (53%) of the alcohol as a clear oil, ^{1}H NMR (300 Mhz; $CDCl_3$): d 1.23 (br, 1H); 1.87 (m, 2H); 2.61 (t, 2H, J = 7.1); 3.66 (t, 2H); 3.80 (s, 3H); 3.83 (s, 6H); 6.40 (s, 2H).

EXAMPLE 17

General procedure for the synthesis of trans-allylic alcohols from acrylic esters. Exemplified for (3,4,5-trimethoxy)phenylprop-2-(E)-enol.

A solution of methyl (3,3,5-trimethoxy)-*trans*-cinnamate (1.35 g; 5.35 mmol) in toluene (25 mL) was cooled to -10°C and treated with a solution of diisobutylaluminum hydride in toluene (11.25 mL of a 1.0 M solution; 11.25 mmol). The reaction mixture was stirred for 3 hrs at 0°C and then quenched with 3 mL of

methanol followed by 1 N HCl until the pH was 1. The reaction mixture was extracted into ethyl acetate and the organic phase was washed with water, dried and concentrated. Purification on a silica gel column eluting with 25% ethyl acetate in hexane furnished 0.96 g (80%) of a thick oil, ^{1}H NMR (360 Mhz; $CDCl_3$): d 3.85 (s, 3H); 3.87 (s, 6H); 4.32 (d, 2H, J = 5.6); 6.29 (dt, 1H, J = 15.8, 5.7), 6.54 (d, 1H, J = 15.8); 6.61 (s, 2H).

EXAMPLE 18

(4,5-dichloro)phenylprop-2-(E)-enol, 89%, ^{1}H NMR (360 Mhz; $CDCl_3$): d 1.55 (s, 1H); 4.34 (d, 2H, J = 4.4); 6.36 (dt, 1H, J = 15.9, 5.3); 6.54 (d, 1H, J = 15.9); 7.20 (dd, 1H, J = 8.3, 1.7); 7.38 (d, 1H, J = 8.3); 7.45 (d, 1H, J = 1.6). This compound was prepared by the method of Example 16 from (3,4,5-trimethoxy)-trans-cinnamate.

EXAMPLE 19

(4,5-methylenedioxy)phenylprop-2-(E)-enol, 80%, ^{1}H NMR (360 Mhz; $CDCl_3$): d 1.59 (br, 1H); 4.29 (br, 2H); 5.96 (s, 2H); 6.20 (dt, 1H, J = 15.8, 5.9); 6.52 (d, 1H, J = 15.8); 6.76 (d, 1H, J = 8.0); 6.82 (dd, 1H, J = 8.0, 1.2); 6.93 (d, 1H, J = 1.2). This compound was prepared by the method of Example 16 from (3,4,5-trimethoxy)-trans-cinnamate.

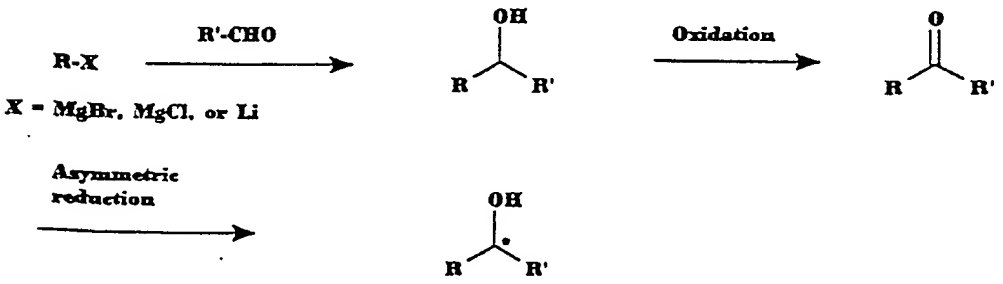
EXAMPLE 20

Phenylprop-2-(E)-enol, 85%, ^{1}H NMR (360 Mhz; $CDCl_3$): d 1.72 (br, 1H); 4.31 (d, 2H, J = 5.7); 6.36

(dt, 1H, J = 15.9, 5.7); 6.61 (d, 1H, J = 15.9); 7.02-7.55 (m, 5H). This compound was prepared by the method of Example 16 from (3,4,5-tri-methoxy)-trans-cinnamate.

EXAMPLE 21

Alcohols containing a substituent at the 1-position of the side chain may be conveniently prepared by addition of appropriate nucleophiles to aldehydes, as described in Scheme III. In cases where optically active substituted alcohols are desired, the racemic alcohols may be oxidized to prochiral ketones and subjected to asymmetric reduction by one of several methods well known to those skilled in the art.



Scheme III

EXAMPLE 22

General procedure for the preparation of 1-substituted alkanols, exemplified for the synthesis of 1,3-diphenylpropanol.

A solution of 2-(bromoethyl)benzene (17.45 g; 94.3 mmol) in 50 mL of dry diethyl ether was added dropwise, under a nitrogen atmosphere, to a stirred slurry of magnesium turnings (2.50 g; 102.8 mmol) in 50 mL of ether. The mixture was initially heated with a heat gun until reflux had become self-sustaining. After the addition was complete, the mixture was heated externally for 30 min to maintain reflux. A solution of 10.01 g (94.3 mmol) of benzaldehyde in 20 mL of ether was then added dropwise, and reflux was continued for 30 min. After cooling, the reaction mixture was poured into 150 mL of saturated ammonium chloride and extracted into ethyl acetate. The crude material obtained upon removal of the solvent was purified on a flash column, eluting with 5% ethyl acetate/hexane to 20% ethyl acetate, to obtain 13.73 g (69%) of the alkanol as a light yellow oil, ^{1}H NMR (360 Mhz; $CDCl_3$): d 1.93-2.30 (m, 3H); 2.70-2.90 (m, 2H); 4.72 (br, 1H); 7.19-7.27 (m, 3H); 7.27-7.36 (m, 3H); 7.36-7.47 (m, 4H).

EXAMPLE 23

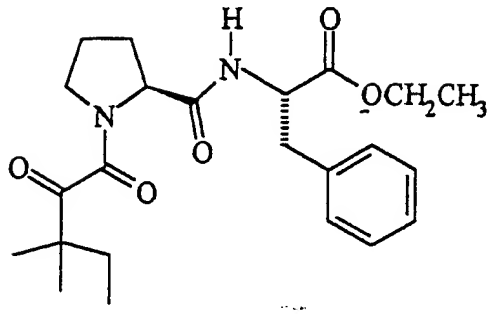
General procedure for conversion of racemic 1-substituted alkanols to optically active 1-substituted alkanols via prochiral ketones. Exemplified for (1R)-1,3-diphenyl-1-propanol.

A solution of racemic 1,3-diphenyl-1-propanol (1.26 g; 5.94 mmol) was dissolved in 10 mL of acetone, and Jones reagent was added until persistence of the

orange color. After stirring for 30 min, the reaction was quenched by adding 2 mL of 2-propanol. The solvent was decanted away from the precipitated solids, which were washed with ethyl acetate. The combined organic fractions were washed with 2 x 20 mL of water, dried and concentrated. The crude product was filtered through a plug of silica gel, eluting with 25% ethyl acetate/hexane, to obtain 1.07 g (86%) of 1,3-diphenylpropanone as a white crystalline solid, ^{1}H NMR (360 Mhz; $CDCl_3$): d 3.09 (t, 2H, J = 8.1); 3.33 (t, 2H, J = 8.1); 7.29 (m, 5H); 7.49 (m, 3H); 7.98 (m, 2H).

A solution of 1,3-diphenylpropanone (1.07 g; 5.09 mmol) in tetrahydrofuran (10 mL) was cooled to -23°C and treated with an asymmetric reducing agent, (+)-B-chlorodiisopinocampheyl-borane (1.80 g; 5.60 mmol) in 20 mL THF, and the resulting solution was allowed to stand overnight at -23°C. After evaporating to dryness, the residue was treated with ether (65 mL) and diethanolamine (1.0 g) and stirred for 3 hrs. The mixture was then filtered to remove solids and concentrated, and the residue was purified using gradient elution (5% ethyl acetate/hexane to 10% ethyl acetate) on a silica gel column to obtain 660 mg (61%) of (1R)-1,3-diphenyl-1-propanol as a crystalline white solid, ^{1}H NMR (360 Mhz; $CDCl_3$): d 1.95-2.15 (m, 3H); 2.59-2.78 (m, 2H); 4.65 (dd, 1H, J = 5.4, 7.8); 7.14-7.35 (m, 10H).

EXAMPLE 24



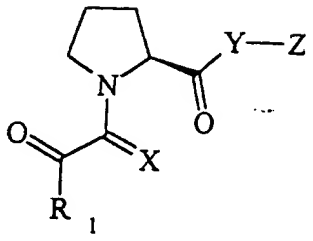
General procedure for the synthesis of prolyl dipeptides, exemplified for 1-[1-(3,3-dimethyl-1,2-dioxopentyl)-L-proline]-L-phenylalanine ethyl ester.

A mixture of (2S)-1-(1,2-dioxo-3,3-dimethylpentyl)-2-pyrrolidinecarboxylic acid (1.17 g; 4.85 mmol), L-phenylalanine ethyl ester hydrochloride (1.23 g; 5.33 mmol), dicyclohexylcarbo- diimide (1.10 g; 5.33 mmol) and 4-dimethylaminopyridine (60 mg (4.85 mmol) in methylene chloride (25 mL) was treated with triethylamine (1 mL; 726 mg; 7.17 mmol) and stirred overnight. The mixture was filtered through Celite to remove solids and concentrated, and the crude material from removal of the solvent was purified on a silica gel column eluting with 30% ethyl acetate/hexane to obtain 2.02 g of 1-[1-(3,3-dimethyl-1,2-dioxopentyl)-L-proline]-L-phenylalanine ethyl ester, 100%, ^{1}H NMR (360 MHz, $CDCl_3$): d 0.87 (t, 3H); 1.16-1.28 (m, 9H); 1.58-1.91 (m, 5H); 2.33 (m, 1H); 3.07-3.20 (m, 2H); 3.38-3.41 (m, 2H); 4.11-4.18 (m, 4H); 4.55 (d, 1H, J = 6.5); 4.78-4.80 (m, 1H); 7.15 (br d, 1H); 7.19 (m, 5H).

The invention being thus described, it will be obvious that the same may be varied in many ways. Such variations are not to be regarded as a departure from the spirit and scope of the invention and all such modification are intended to be included within the scope of the following claims.

What is claimed is:

1. A neurotrophic compound of the formula:



where

R_1 is a C_1-C_9 straight or branched chain alkyl or alkenyl group optionally substituted with C_3-C_8 cycloalkyl, C_3 or C_5 cycloalkyl, C_5-C_7 cycloalkenyl, or Ar_1, where said alkyl, alkenyl, cycloalkyl or cycloalkenyl groups may be optionally substituted with C_1-C_4 alkyl, C_1-C_4 alkenyl, or hydroxy, and where Ar_1 is selected from the group consisting of 1-napthyl, 2-napthyl, 2-indolyl, 3-indolyl, 2-furyl, 3-furyl, 2-thienyl, 3-thienyl, 2-, 3-, or 4-pyridyl, or phenyl, having one to three substituents which are independently selected from the group consisting of hydrogen, halo, hydroxyl, nitro, trifluoromethyl, C_1-C_6 straight or branched alkyl or alkenyl, C_1-C_4 alkoxy or C_1-C_4

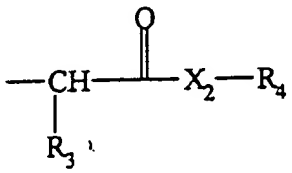
alkenyloxy, phenoxy, benzyloxy, and amino;

X is oxygen, sulfur, methylene (CH_2), or H_2;

Y is oxygen or NR_2, where R_2 is hydrogen or C_1-C_6 alkyl; and

Z is a C2-C6 straight or branched chain alkyl or alkenyl, wherein the alkyl chain is substituted in one or more positions with Ar_1 as defined above, C_3-C_8 cycloalkyl, cycloalkyl connected by a C_1-C_6 straight or unbranched alkyl or alkenyl chain, or Ar_2 where Ar_2 is selected from the group consisting of 2-indolyl, 3-indolyl, 2-furyl, 3-furyl, 2-thiazolyl, 2-thienyl, 3-thienyl, 2-, 3-, or 4-pyridyl, or phenyl, having one to three substituents which are independently selected from the group consisting of hydrogen, halo, hydroxyl, nitro, trifluoromethyl, C_1-C_6 straight or branched alkyl or alkenyl, C_1-C_4 alkoxy or C_1-C_4 alkenyloxy, phenoxy, benzyloxy, and amino;

Z may also be the fragment:



where

R_3 is selected from the group consisiting of straight or branched alkyl C_1-C_8 optionally substituted with C_3-C_8 cycloalkyl, or Ar_1 as defined above;

X_2 is O or NR_5, where R_5 is selected from the group consisting of hydrogen, C_1-C_6 straight or branched alkyl and alkenyl;

R_4 is selected from the group consisting of phenyl, benzyl, C_1-C_5 straight or branched alkyl or alkenyl, and C_1-C_5 straight or branched alkyl or alkenyl substituted with phenyl; or pharmaceutically acceptable salts or hydrates thereof.

2. The neurotrophic compound of claim 1, which has an affinity for FKBP-type immunophilins.

3. The neurotrophic compound of claim 2, where the FKBP-type immunophilin is FKBP-12.

4. The neurotrophic compound of claim 1, capable of inhibiting rotamase activity.

5. The neurotrophic compound of claim 1, where Z and R_1 are lipophilic groups.

6. The neurotrophic compound according to claim 1 that is selected from the group consisting of

3-phenyl-1-propyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate,

3-phenyl-1-prop-2-(E)-enyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate,

3-(3,4,5-trimethoxyphenyl)-1-propyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate,

3-(3,4,5-trimethoxyphenyl)-1-prop-2-(E)-enyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate,

3-(4,5-dichlorophenyl)-1-propyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate,

3-(4,5-dichlorophenyl)-1-prop-2-(E)-enyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate,

3-(4,5-methylenedioxyphenyl)-1-propyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate,

3-(4,5-methylenedioxyphenyl)-1-prop-2-(E)-enyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate,

3-cyclohexyl-1-propyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate,

3-cyclohexyl-1-prop-2-(E)-enyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate,

(1R)-1,3-diphenyl-1-propyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate,

(1R)-1,3-diphenyl-1-prop-2-(E)-enyl (2S)-1-(3,3-

dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate,

(1R)-1-cyclohexyl-3-phenyl-1-propyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate,

(1R)-1-cyclohexyl-3-phenyl-1-prop-2-(E)-enyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate,

(1R)-1-(4,5-dichlorophenyl)-3-phenyl-1-propyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate,

3-phenyl-1-propyl (2S)-1-(1,2-dioxo-2-cyclohexyl)ethyl-2-pyrrolidinecarboxylate,

3-phenyl-1-propyl (2S)-1-(1,2-dioxo-4-cyclohexyl)butyl-2-pyrrolidinecarboxylate,

3-phenyl-1-propyl (2S)-1-(1,2-dioxo-2-[2-furanyl])ethyl-2-pyrrolidinecarboxylate,

3-phenyl-1-propyl (2S)-1-(1,2-dioxo-2-[2-thienyl])ethyl-2-pyrrolidinecarboxylate,

3-phenyl-1-propyl (2S)-1-(1,2-dioxo-2-[2-thiazolyl])ethyl-2-pyrrolidinecarboxylate,

3-phenyl-1-propyl (2S)-1-(1,2-dioxo-2-phenyl)ethyl-2-pyrrolidinecarboxylate,

1,7-diphenyl-4-heptyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate,

3-Phenyl-1-propyl (2S)-1-(3,3-dimethyl-1,2-dioxo-4-hydroxybutyl)-2-pyrrolidinecarboxylate,

3-phenyl-1-propyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxamide,

1-[1-(3,3-dimethyl-1,2-dioxopentyl)-L-proline]-L-

phenylalanine ethyl ester,

1-[1-(3,3-dimethyl-1,2-dioxopentyl)-L-proline]-L-leucine ethyl ester,

1-[1-(3,3-dimethyl-1,2-dioxopentyl)-L-proline]-L-phenylglycine ethyl ester,

1-[1-(3,3-dimethyl-1,2-dioxopentyl)-L-proline]-L-phenylalanine phenyl ester,

1-[1-(3,3-dimethyl-1,2-dioxopentyl)-L-proline]-L-phenylalanine benzyl ester, and

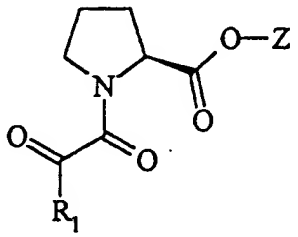
1-[1-(3,3-dimethyl-1,2-dioxopentyl)-L-proline]-L-isoleucine ethyl ester.

7. A pharmaceutical composition comprising a neurotrophically effective amouht of the compound of claim 1 and a pharmaceutically acceptable carrier.

8. A method of stimulating growth of damaged peripheral nerves, which comprises:

administering to damaged peripheral nerves the neurotrophic compound of claim 1 in sufficient amounts to stimulate the growth of said nerves.

9. A neurotrophic compound of the formula:



where

R_1 is a C_1-C_9 straight or branched chain alkyl or alkenyl group optionally substituted with C_3-C_8 cycloalkyl, C_3 or C_5 cycloalkyl, C_5-C_7 cycloalkenyl, or Ar_1, where said alkyl, alkenyl, cycloalkyl or cycloalkenyl groups may be optionally substituted with C_1-C_4 alkyl, C_1-C_4 alkenyl, or hydroxy, and where Ar_1 is selected from the group consisting of 1-napthyl, 2-napthyl, 2-indolyl, 3-indolyl, 2-furyl, 3-furyl, 2-thiazolyl, 2-thienyl, 3-thienyl, 2-, 3-, or 4-pyridyl, or phenyl, having one to three substituents which are independently selected from the group consisting of hydrogen, halo, hydroxyl, nitro, trifluoromethyl, C_1-C_6 straight or branched alkyl or alkenyl, C_1-C_4 alkoxy or C_1-C_4 alkenyloxy, phenoxy, benzyloxy, and amino;

Z is a C_2-C_6 straight or branched chain alkyl or alkenyl, wherein the alkyl chain is substituted in one or more positions with Ar_1 as defined above, C_3-C_8 cycloalkyl, cycloalkyl connected by a C_1-C_6 straight or unbranched alkyl or alkenyl chain, or Ar_2 where Ar_2 is selected from the group consisting of 2-indolyl, 3-indolyl, 2-furyl, 3-furyl, 2-thiazolyl, 2-thienyl, 3-thienyl, 2-, 3-, or 4-pyridyl, or phenyl,

having one to three substituents which are independently selected from the group consisting of hydrogen, halo, hydroxyl, nitro, trifluoromethyl, C_1-C_6 straight or branched alkyl or alkenyl, C_1-C_4 alkoxy or C_1-C_4 alkenyloxy, phenoxy, benzyloxy, and amino; or pharmaceutically acceptable salts or hydrates thereof.

10. The neurotrophic compound of claim 9, wherein R_1 is selected from the group consisting of C_1-C_9 straight or branched chain alkyl, 2-cyclohexyl, 4-cyclohexyl, 2-furanyl, 2- thienyl, 2-thiazolyl, and 4-hydroxybutyl.

11. The neurotrophic compound of claim 9 having an affinity for FKBP-type immunophilins.

12. The neurotrophic compounds of claim 11, where the FKBP-type immunophilin is FKBP-12.

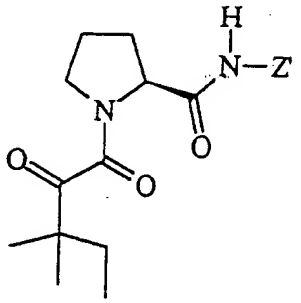
13. The neurotrophic compound of claim 9, capable of inhibiting rotamase activity.

14. The neurotrophic compound of claim 9, where Z and R_1 are liphophilic groups.

15. A pharmaceutical composition comprising a neurotrophically effective amount of the compound of

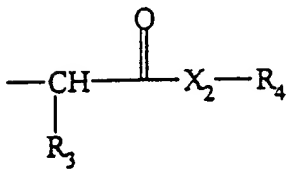
claim 9 and a pharmaceutically acceptable carrier.

16. A neurotrophic compound of the formula:



where

Z' is the fragment:



where

R_3 is selected from the group consisting of straight or branched alkyl C_1-C_8 optionally substituted with C_3-C_8 cycloalkyl, or Ar_1 as defined above, and unsubstituted Ar_1;

X_2 is O or NR_5, where R_5 is selected from the group consisting of hydrogen, C_1-C_6 straight or branched alkyl and alkenyl;

R_4 is selected from the group consisting of phenyl, benzyl, C_1-C_5 straight or branched alkyl or alkenyl, and C_1-C_5 straight or branched alkyl or alkenyl substituted with phenyl; or pharmaceutically acceptable salts or hydrates thereof.

17. The neurotrophic compound of claim 16, having an affinity for FKBP-type immunophilins.

18. The neurotrophic compound of claim 17, where the FKBP-type immunophilin is FKBP-12.

19. The neurotrophic compound of claim 16, capable of inhibiting rotamase activity.

20. The neurotrophic compound of claim 16, where Z' is a lipophilic group.

21. A pharmaceutical composition comprising a neurotrophically effective amount of the compound of claim 16 and a pharmaceutically acceptable carrier.

22. A neurotrophic compound having an affinity for FKBP-type immunophilins wherein the immunophilin exhibits rotamase activity and the neurotrophic compound inhibits the rotamase activity of the immunophilin.

23. The neurotrophic compound of claim 22, wherein the FKBP-type immunophilin is FKBP-12.

24. A method of treating a neurological disorder in an animal comprising administering a therapeutically effective amount of a compound having an affinity for FKBP-type immunophilins wherein the immunophilin exhibits rotamase activity and the neurotrophic compound inhibits the rotamase activity of the immunophilin.

25. The method of claim 24, wherein the FKBP-type immunophilin is FKBP-12.

26. The method of claim 24, wherein the neurological disorder is selected from the group consisting of peripheral neuropathies, and neurological pathologies related to neurodegeneration.

27. The method of claim 24, wherein the neurological disorder is Alzheimer's disease.

28. The method of claim 24, wherein the neurological disorder is Parkinson's disease.

29. The method of claim 24, wherein the neurological disorder is amyotrophic lateral sclerosis.

30. A method of promoting neuronal regeneration and growth in mammals, comprising administering to a subject an effective amount of a neurotrophic compound having an affinity for FKBP-type immunophilins wherein the immunophilin exhibits rotamase activity and the neurotrophic compound inhibits the rotamase activity of the immunophilin.

31. The method of claim 30, wherein the FKBP-type immunophilin is FKBP-12.

32. A method of preventing neurodegeneration in an animal comprising administering an effective amount of a compound having an affinity for FKBP-type immunophilins wherein the immunophilin exhibits rotamase activity and the neurotrophic compound inhibits the rotamase activity of the immunophilin.

33. The method of claim 32, wherein the FKBP-type immunophilin is FKBP-12.

ABSTRACT OF THE DISCLOSURE

This invention relates to neurotrophic compounds having an affinity for FKBP-type immunophilins, their preparation and use as inhibitors of the enzyme activity associated with immunophilin proteins, and particularly inhibitors of peptidyl-prolyl isomerase or rotamase enzyme activity.

08/479436

PATENT APPLICATION SERIAL NO._____

U.S. DEPARTMENT OF COMMERCE
PATENT AND TRADEMARK OFFICE
FEE RECORD SHEET

Earliest priority application for '298

(See ¶ bridging pages 6-7 of Request.)

PTO-1556
(5/87)

PTO-103X
(Rev. 8-95)

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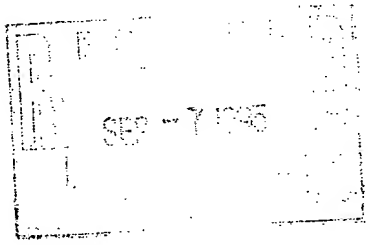
TITLE
SMALL MOLECULE INHIBITORS OF ROTAMASE ENZYME

PRELIMINARY CLASS: 514



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Attorney Docket 22762

Title:

SMALL MOLECULE INHIBITORS OF ROTAMASE ENZYME ACTIVITY

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BACKGROUND OF THE INVENTION

1. Field of the Invention

This invention relates to neurotrophic compounds having an affinity for FKBP-type immunophilins, their preparation and use as inhibitors of the enzyme activity associated with immunophilin proteins, and particularly inhibitors of peptidyl-prolyl isomerase or rotamase enzyme activity.

2. Description of the Prior Art

The term immunophilin refers to a number of proteins that serve as receptors for the principal immunosuppressant drugs, cyclosporin A (CsA), FK506, and rapamycin. Known classes of immunophilins are cyclophilins, and FK506 binding proteins, such as FKBP. Cyclosporin A binds to cyclophilin while FK506 and rapamycin bind to FKBP. These immunophilin-drug complexes interface with a variety of intracellular signal transduction systems, especially in the immune system and the nervous system.

Immunophilins are known to have peptidyl-prolyl isomerase (PPIase) or rotamase enzyme activity. It has been determined that rotamase activity has a role in the catalyzation of the interconversion of the cis and trans isomer of immunophilin proteins.

Immunophilins were originally discovered and studied in immune tissue. It was initially postulated by those skilled in the art that inhibition of the immunophilins rotamase activity leads to the inhibition

of T-cell proliferation, thereby causing the immunosuppressive action exhibited by immunosuppressive drugs such as cyclosporin A, FK506, and rapamycin. Further study has shown that the inhibition of rotamase activity, in and of itself, is not sufficient for immunosuppressant activity. Schreiber et al., *Science*, 1990 vol. 250 pp. 556-559. It has been shown that the immunophilin-drug complexes interact with ternary protein targets as their mode of action. Schreiber et al., *Cell*, 1991, vol. 66, pp. 807-815. In the case of FKBP-FK506 and FKBP-CsA, the drug-immunophilin complexes bind to the enzyme calcineurin, inhibitory T-cell receptor signalling leading to T-cell proliferation. Similarly, the complex of rapamycin and FKBP interacts with the RAFT1/FRAP protein and inhibits signalling from the IL-2 receptor.

Immunophilins have been found to be present at high concentrations in the central nervous system. Immunophilins are enriched 10-50 times more in the central nervous system than in the immune system. Within neural tissues, immunophilins appear to influence neuronal process extension, nitric oxide synthesis, and neurotransmitter release.

It has been found that picomolar concentrations of an immunosuppressant such as FK506 and rapamycin stimulate neurite out growth in PC12 cells and sensory nervous, namely dorsal root ganglion cells (DRGs). Lyons et al., *Proc. of Natl. Acad. Sci.*, 1994 vol. 91,

pp. 3191-3195. In whole animal experiments, FK506 has been shown to stimulate nerve regeneration following facial nerve injury and results in functional recovery in animals with sciatic nerve lesions.

Surprisingly, it has been found that drugs with a high affinity for FKBP are potent rotamase inhibitors causing a neurotrophic effect. Lyons et al. These findings suggest the use of immunosuppressants in treating various peripheral neuropathies and enhancing neuronal regrowth in the central nervous system (CNS). Studies have demonstrated that neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis (ALS) may occur due to the loss, or decreased availability, of a neurotrophic substance specific for a particular population of neurons affected in the disorder.

Several neurotrophic factors effecting specific neuronal populations in the central nervous system have been identified. For example, it has been hypothesized that Alzheimer's disease results from a decrease or loss of nerve growth factor (NGF). It has thus been proposed to treat Alzheimer's patients with exogenous nerve growth factor or other neurotrophic proteins such as brain derived nerve factor (BDNF), glial derived nerve factor, ciliary neurotrophic factor, and neurotropin-3 to increase the survival of degenerating neuronal populations.

Clinical application of these proteins in various neurological disease states is hampered by difficulties in the delivery and bioavailability of large proteins to nervous system targets. By contrast, immunosuppressant drugs with neurotrophic activity are relatively small and display excellent bioavailability and specificity. However, when administered chronically, immunosuppressants exhibit a number of potentially serious side effects including nephrotoxicity, such as impairment of glomerular filtration and irreversible interstitial fibrosis (Kopp et al., 1991, J. Am. Soc. Nephrol. 1:162); neurological deficits, such as involuntary tremors, or non-specific cerebral angina such as non-localized headaches (De Groen et al., 1987, N. Engl. J. Med. 317:861); and vascular hypertension with complications resulting therefrom (Kahan et al., 1989 N. Engl. J. Med. 321: 1725).

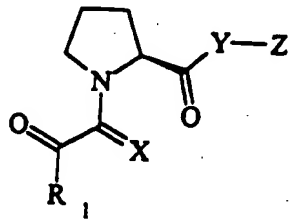
In order to prevent the side effects associated with use of the immunosuppressant compounds, the present invention provides non-immunosuppressive compounds containing small molecule FKBP rotamase inhibitors for promoting neuronal growth and regeneration in various neuropathological situations where neuronal repair can be facilitated including peripheral nerve damage by physical injury or disease state such as diabetes, physical damage to the central nervous system (spinal cord and brain) brain damage

associated with stroke, and for the treatment of neurological disorders relating to neurodegeneration, including Parkinson's disease, Alzheimer's disease, and amyotrophic lateral sclerosis.

SUMMARY OF THE INVENTION

The present invention relates to a novel class of neurotrophic compounds having an affinity for FKBP-type immunophilins. Once bound to this protein the neurotrophic compounds are potent inhibitors of the enzyme activity associated with immunophilin proteins and particularly rotamase enzyme activity, thereby stimulating neuronal regeneration and outgrowth. A key feature of the compounds of the present invention is that they do not exert any significant immunosuppressive activity in addition to their neurotrophic activity.

A preferred embodiment of this invention is a neurotrophic compound of the formula:



where

R_1 is selected from the group consisting of a C_1-C_9 straight or branched chain alkyl or alkenyl group optionally substituted with C_3-C_6 cycloalkyl, C_3 or C_5 cycloalkyl, C_5-C_7 cycloalkenyl, Ar_1, where said alkyl, alkenyl, cycloalkyl or cycloalkenyl groups may be optionally substituted with C_1-C_4 alkyl, C_1-C_4 alkenyl, or hydroxy, where Ar_1 is selected from the group consisting of 1-napthyl, 2-napthyl, 2-indolyl, 3-indolyl, 2-furyl, 3-furyl, 2-thiazolyl, 2-thienyl, 3-thienyl, 2-,3-, 4-pyridyl, and phenyl, having one to three substituents which are independently selected from the group consisting of hydrogen, halo, hydroxyl, nitro, trifluoromethyl, C_1-C_6 straight or branched alkyl or alkenyl, C_1-C_4 alkoxy or C_1-C_4 alkenyloxy, phenoxy, benzyloxy, and amino;

X is selected from the group consisting of oxygen, sulfur, methylene (CH_2), or H_2;

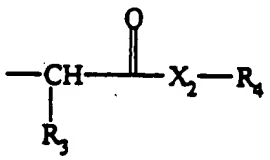
Y is selected from the group consisting of oxygen or NR_2, where R_2 is hydrogen or C_1-C_6 alkyl; and

Z is selected from the group consisting of C_2-C_6 straight or branched chain alkyl or alkenyl,

wherein the alkyl chain is substituted in one or more positions with Ar_1 as defined above, C_3-C_8 cycloalkyl, cycloalkyl connected by a C_1-C_6

straight or unbranched alkyl or alkenyl chain, and Ar_2 where Ar_2 is selected from the group consisting of 2-indolyl, 3-indolyl, 2-furyl, 3-furyl, 2- thiazolyl, 2-thienyl, 3-thienyl, 2-, 3-, or 4-pyridyl, and phenyl, having one to three substituents which are independently selected from the group consisting of hydrogen, halo, hydroxyl, nitro, trifluoromethyl, C_1-C_6 straight or branched alkyl or alkenyl, C_1-C_4 alkoxy or C_1-C_4 alkenyloxy, phenoxy, benzyloxy, and amino;

Z may also be the fragment:



where

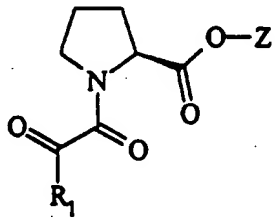
R_3 is selected from the group consisting of straight or branched alkyl C_1-C_8 optionally substituted with C_3-C_6 cycloalkyl, or Ar_1 as defined above, and unsubstituted Ar_1;

X_2 is O or NR_5, where R_5 is selected from the group consisting of hydrogen, C_1-C_6 straight or branched alkyl and alkenyl;

R_4 is selected from the group consisting of phenyl, benzyl, C_1-C_5 straight or branched alkyl or alkenyl, and C_1-C_5 straight or

branched alkyl or alkenyl substituted with phenyl; or pharmaceutically acceptable salts or hydrates thereof.

Another preferred embodiment of this invention is a neurotrophic compound of the formula:



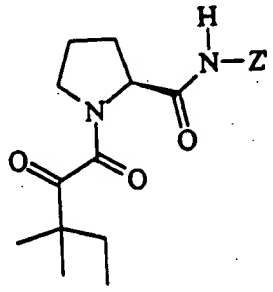
where

R_1 is a C_1-C_9 straight or branched chain alkyl or alkenyl group optionally substituted with C_3-C_8 cycloalkyl, C_3 or C_5 cycloalkyl, C_5-C_7 cycloalkenyl, or Ar_1, where said alkyl, alkenyl, cycloalkyl or cycloalkenyl groups may be optionally substituted with C_1-C_4 alkyl, C_1-C_4 alkenyl, or hydroxy, and where Ar_1 is selected from the group consisting of 1-napthyl, 2-napthyl, 2-indolyl, 3-indolyl, 2-furyl, 3-furyl, 2-thiazolyl, 2-thienyl, 3-thienyl, 2-,3-, or 4-pyridyl, or phenyl, having one to three substituents which are independently selected from the group consisting of hydrogen, halo, hydroxyl, nitro, trifluoromethyl, C_1-C_6 straight or branched alkyl or alkenyl, C_1-C_4 alkoxy or C_1-

C_4 alkenyloxy, phenoxy, benzyloxy, and amino;

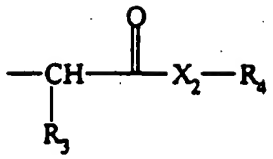
Z is a C_2-C_6 straight or branched chain alkyl or alkenyl, wherein the alkyl chain is substituted in one or more positions with Ar_1 as defined above, C_3-C_8 cycloalkyl, cycloalkyl connected by a C_1-C_6 straight or unbranched alkyl or alkenyl chain, or Ar_2 where Ar_2 is selected from the group consisting of 2-indolyl, 3-indolyl, 2-furyl, 3-furyl, 2- thiazolyl, 2-thienyl, 3-thienyl, 2-, 3-, or 4-pyridyl, or phenyl, having one to three substituents which are independently selected from the group consisting of hydrogen, halo, hydroxyl, nitro, trifluoromethyl, C_1-C_6 straight or branched alkyl or alkenyl, C_1-C_4 alkoxy or C_1-C_4 alkenyloxy, phenoxy, benzyloxy, and amino; or pharmaceutically acceptable salts or hydrates thereof.

Another preferred embodiment of this invention is a neurotrophic compound of the formula:



where

Z' is the fragment:



where

R_3 is selected from the group consisting of straight or branched alkyl C_1-C_8 optionally substituted with C_3-C_8 cycloalkyl, or Ar_1 as defined above, and unsubstituted Ar_1;

X_2 is O or NR_5, where R_5 is selected from the group consisting of hydrogen, C_1-C_6 straight or branched alkyl and alkenyl;

R_4 is selected from the group consisting of phenyl, benzyl, C_1-C_5 straight or branched alkyl or alkenyl, and C_1-C_5 straight or branched alkyl or alkenyl substituted with phenyl; or pharmaceutically acceptable salts or hydrates thereof.

Another preferred embodiment of the invention is a neurotrophic compound having an affinity for FKBP-type immunophilins which inhibit the rotamase activity of the immunophilin.

Another preferred embodiment of the present invention is a method for treating a neurological disorder in an animal comprising administering a therapeutically effective amount of a compound having an affinity for FKBP-type immunophilins which inhibits the rotamase activity of the immunophilin.

Another preferred embodiment of the invention is a method of promoting neuronal regeneration and growth in mammals, comprising administering to a mammal an effective amount of a neurotrophic compound having an affinity for FKBP-type immunophilins which inhibits the rotamase activity of the immunophilin.

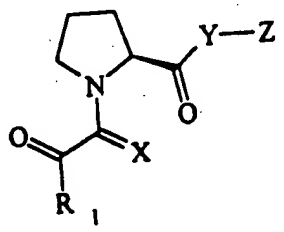
Yet another preferred embodiment of the invention is a method of preventing neurodegeneration in an animal comprisihg administering to an animal an effective amount of a neurotrophic compound having an affinity for FKBP-type immunophilins which inhibits rotamase activity of the immunophilin.

DETAILED DESCRIPTION OF THE INVENTION

The novel neurotrophic compounds of this invention are relatively small molecules in relation to other known compounds which bind to FKBP-type immunophilins, such as rapamycin, FK506, and cyclosporin.

The neurotrophic compounds of this invention have an affinity for the FK506 binding proteins such as FKBP-12. When the neurotrophic compounds of the invention are bound to the FKBP, they have been found

to unexpectedly inhibit the prolyl- peptidyl cis-trans isomerase activity, or rotamase activity of the binding protein and stimulate neurite growth, while not exhibiting an immunosuppressant effect. More particularly, this invention relates to a novel class of neurotrophic compounds represented by the formula:



I

where

R_1 is a C_1-C_9 straight or branched chain alkyl or alkenyl group optionally substituted with C_3-C_8 cycloalkyl, C_3 or C_5 cycloalkyl, C_5-C_7 cycloalkenyl, or Ar_1, where said alkyl, alkenyl, cycloalkyl or cycloalkenyl groups may be optionally substituted with C_1-C_4 alkyl, C_1-C_4 alkenyl, or hydroxy, and where Ar_1 is selected from the group consisting of 1-napthyl, 2-napthyl, 2-indolyl, 3-indolyl, 2-furyl, 3-furyl, 2- thiazolyl, 2-thienyl, 3-thienyl, 2-, 3-, or 4-pyridyl, or phenyl, having one to three substituents which are independently selected from the group

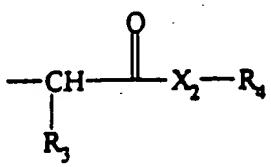
consisting of hydrogen, halo, hydroxyl, nitro, trifluoromethyl, C_1-C_6 straight or branched alkyl or alkenyl, C_1-C_4 alkoxy or C_1-C_4 alkenyloxy, phenoxy, benzyloxy, and amino;

X is oxygen, sulfur, methylene (CH_2), or H_2;

Y is oxygen or NR_2, where R_2 is hydrogen or C_1-C_6 alkyl; and

Z is a C_2-C_6 straight or branched chain alkyl or alkenyl, wherein the alkyl chain is substituted in one or more positions with Ar_1 as defined above, C_3-C_8 cycloalkyl, cycloalkyl connected by a C_1-C_6 straight or unbranched alkyl or alkenyl chain, or Ar_2 where Ar_2 is selected from the group consisting of 2-indolyl, 3-indolyl, 2-furyl, 3-furyl, 2- thiazolyl, 2-thienyl, 3-thienyl, 2-, 3-, or 4-pyridyl, or phenyl, having one to three substituents which are independently selected from the group consisting of hydrogen, halo, hydroxyl, nitro, trifluoromethyl, C_1-C_6 straight or branched alkyl or alkenyl, C_1-C_4 alkoxy or C1-C4 alkenyloxy, phenoxy, benzyloxy, and amino;

Z may also be the fragment:



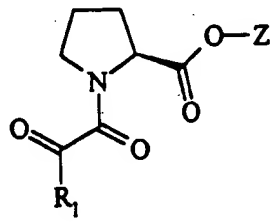
where

R_3 is selected from the group consisting of straight or branched alkyl C_1-C_8 optionally substituted with C_3-C_8 cycloalkyl, or Ar_1 as defined above, and unsubstituted Ar_1;

X_2 is O or NR_5, where R_5 is selected from the group consisting of hydrogen, C_1-C_6 straight or branched alkyl and alkenyl;

R_4 is selected from the group consisting of phenyl, benzyl, C_1-C_5 straight or branched alkyl or alkenyl, and C_1-C_5 straight or branched alkyl or alkenyl substituted with phenyl; or pharmaceutically acceptable salts or hydrates thereof.

Preferred compounds have the following formula:



II

where

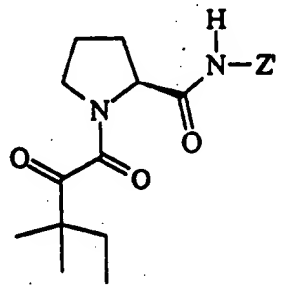
R_1 is a C_1-C_9 straight or branched chain alkyl or alkenyl group optionally substituted with C_3-C_8 cycloalkyl, C_3 or C_5 cycloalkyl, C_5-C_7

cycloalkenyl, or Ar_1, where said alkyl, alkenyl, cycloalkyl or cycloalkenyl groups may be optionally substituted with C_1-C_4 alkyl, C_1-C_4 alkenyl, or hydroxy, and where Ar_1 is selected from the group consisting of 1-napthyl, 2-napthyl, 2-indolyl, 3-indolyl, 2-furyl, 3-furyl, 2- thiazolyl, 2-thienyl, 3-thienyl, 2-, 3-, or 4-pyridyl, or phenyl, having one to three substituents which are independently selected from the group consisting of hydrogen, halo, hydroxyl, nitro, trifluoromethyl, C_1-C_6 straight or branched alkyl or alkenyl, C_1-C_4 alkoxy or C_1-C_4 alkenyloxy, phenoxy, benzyloxy, and amino;

Z is a C_2-C_6 straight or branched chain alkyl or alkenyl, wherein the alkyl chain is substituted in one or more positions with Ar_1 as defined above, C3-C8 cycloalkyl, cycloalkyl connected by a C_1-C_6 straight or unbranched alkyl or alkenyl chain, or Ar_2 where Ar_2 is selected from the group consisting of 2-indolyl, 3-indolyl, 2-furyl, 3-furyl, 2- thiazolyl, 2-thienyl, 3-thienyl, 2-, 3-, or 4-pyridyl, or phenyl, having one to three substituents which are independently selected from the group consisting of hydrogen, halo, hydroxyl, nitro, trifluoromethyl, C_1-C_6 straight or

branched alkyl or alkenyl, C_1-C_4 alkoxy or C_1-C_4 alkenyloxy, phenoxy, benzyloxy, and amino; or pharmaceutically acceptable salts or hydrates thereof.

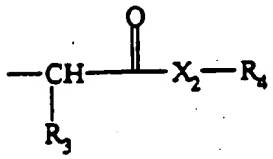
In another preferred embodiment novel compounds of this invention are represented by the formula:



III

where

Z' is the fragment:



where

R_3 is selected from the group consisting of straight or branched alkyl C_1-C_6 optionally substituted with C_3-C_8 cycloalkyl, or Ar_1 as defined above, or unsubstituted Ar_1;

X_2 is O or NR_5, where R_5 is selected from the group consisting of hydrogen, C_1-C_6 straight or branched alkyl and alkenyl;

R_4 is selected from the group consisting of phenyl, benzyl, C_1-C_5 straight or branched alkyl or alkenyl, and C_1-C_5 straight or branched alkyl or alkenyl substituted with phenyl; or pharmaceutically acceptable salts or hydrates thereof.

The compounds of this invention exist as stereoisomeric forms, either enantiomers or diastereoisomers. The stereochemistry at position 1 (Formula 1) is R or S, with S preferred. Included within the scope of the invention are the enantiomers, the racemic form, and diastereoisomeric mixtures. Enantiomers as well as diastereoisomers can be separated by methods known to those skilled in the art.

It is known that immunophilins such as FKBP preferentially recognize peptide substrates containing Xaa-Pro-Yaa motifs, where Xaa and Yaa are lipophilic amino acid residues. Schreiber et al. 1990 *J. Org. Chem.* 55, 4984-4986; Harrison and Stein, 1990 *Biochemistry*, 29, 3813-3816. Thus modified prolyl peptidomimetic compounds bearing lipophilic substituents should bind with high affinity to the hydrophobic core of the FKBP active site and inhibit its rotamase activity.

Preferred compounds of the invention include:

3-phenyl-1-propyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate,

3-phenyl-1-prop-2-(E)-enyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate,

3-(3,4,5-trimethoxyphenyl)-1-propyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate,

3-(3,4,5-trimethoxyphenyl)-1-prop-2-(E)-enyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate,

3-(4,5-dichlorophenyl)-1-propyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate,

3-(4,5-dichlorophenyl)-1-prop-2-(E)-enyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate,

3-(4,5-methylenedioxyphenyl)-1-propyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate,

3-(4,5-methylenedioxyphenyl)-1-prop-2-(E)-enyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate,

3-cyclohexyl-1-propyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate,

3-cyclohexyl-1-prop-2-(E)-enyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate,

(1R)-1,3-diphenyl-1-propyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate,

(1R)-1,3-diphenyl-1-prop-2-(E)-enyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate,

(1R)-1-cyclohexyl-3-phenyl-1-propyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate,

(1R)-1-cyclohexyl-3-phenyl-1-prop-2-(E)-enyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate,

(1R)-1-(4,5-dichlorophenyl)-3-phenyl-1-propyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate,

3-phenyl-1-propyl (2S)-1-(1,2-dioxo-2-cyclohexyl)ethyl-2-pyrrolidinecarboxylate,

3-phenyl-1-propyl (2S)-1-(1,2-dioxo-4-cyclohexyl)butyl-2-pyrrolidinecarboxylate,

3-phenyl-1-propyl (2S)-1-(1,2-dioxo-2-[2-furanyl])ethyl-2-pyrrolidinecarboxylate,

3-phenyl-1-propyl (2S)-1-(1,2-dioxo-2-[2-thienyl])ethyl-2-pyrrolidinecarboxylate,

3-phenyl-1-propyl (2S)-1-(1,2-dioxo-2-[2-thiazolyl])ethyl-2-pyrrolidinecarboxylate,

3-phenyl-1-propyl (2S)-1-(1,2-dioxo-2-phenyl)ethyl-2-pyrrolidinecarboxylate,

1,7-diphenyl-4-heptyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate,

3-Phenyl-1-propyl (2S)-1-(3,3-dimethyl-1,2-dioxo-4-hydroxybutyl)-2-pyrrolidinecarboxylate,

3-phenyl-1-propyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxamide,

1-[1-(3,3-dimethyl-1,2-dioxopentyl)-L-proline]-L-phenylalanine ethyl ester,

1-[1-(3,3-dimethyl-1,2-dioxopentyl)-L-proline]-L-leucine ethyl ester,

1-[1-(3,3-dimethyl-1,2-dioxopentyl)-L-proline]-L-phenylglycine ethyl ester,

1-[1-(3,3-dimethyl-1,2-dioxopentyl)-L-proline]-L-phenylalanine phenyl ester,

1-[1-(3,3-dimethyl-1,2-dioxopentyl)-L-proline]-L-phenylalanine benzyl ester, and

1-[1-(3,3-dimethyl-1,2-dioxopentyl)-L-proline]-L-isoleucine ethyl ester.

The compounds of the present invention can be used in the form of salts derived from inorganic or organic acids and bases. Included among such acid salts are the following: acetate, adipate, alginate, aspartate, benzoate, benzenesulfonate, bisulfate butyrate, citrate, camphorate, camphorsulfonate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, fumarate, glucoheptanoate, glycerophosphate, hemissulfate heptanoate, hexanoate, hydrochloride, hydrobromide, hydroiodide, 2-hydroxyethanesulfonate, lactate, maleate, methanesulfonate, 2-naphthalensulfonate, nicotinate, oxalate, pamoate, pectinate, propionate, succinate, tartrate, thiocyanate, tosylate and undecanoate. Base salts include ammonium salts, alkali metal salts such as sodium and potassium salts, alkaline earth metal salts such as calcium and magnesium salts, salt with organic bases such as dicyclohexylamine salts, N-methyl-D-glucamine, and salts with amino acids such as arginine, lysine, and so forth. Also, the basic

nitrogen-containing groups can be quarternized with such agents as lower alkyl halides, such as methyl, ethyl, propyl, and butyl chloride, bromides and iodides; dialkyl sulfates like dimethyl, diethyl, dibutyl and diamyl sulfates, long chain halides such as decyl, lauryl, myristyl and stearyl chlorides, bromides and iodides, aralkyl halides like benzyl and phenethyl bromides and others. Water or oil-soluble or dispersible products are thereby obtained.

The neurotrophic compounds of this invention can be periodically administered to a patient undergoing treatment for neurological disorders or for other reasons in which it is desirable to stimulate neuronal regeneration and growth, such as in various peripheral neuropathic and neurological disorders relating to neurodegeneration. The compounds of this invention can also be administered to mammals other than humans for treatment of various mammalian neurological disorders.

The novel compounds of the present invention are potent inhibitors of rotamase activity and possess an excellent degree of neurotrophic activity. This activity is useful in the stimulation of damaged neurons, the promotion of neuronal regeneration, the prevention of neurodegeneration, and in the treatment of several neurological disorders known to be associated with neuronal degeneration and peripheral neuropathies. The neurological disorders that may be treated include but are not limited to: trigeminal

neuralgia, glossopharyngeal neuralgia, Bell's Palsy, myasthenia gravis, muscular dystrophy, amyotrophic lateral sclerosis, progressive muscular atrophy, progressive bulbar inherited muscular atrophy, herniated, ruptured or prolapsed invertabrae disk syndromes, cervical spondylosis, plexus disorders, thoracic outlet destruction syndromes, peripheral neuropathic such as those caused by lead, dapsone, ticks, prophyria, or Gullain-Barré syndrome, Alzheimer's disease, and Parkinson's disease.

For these purposes the compounds of the present invention may be administered orally, parenterally, by inhalation spray, topically, rectally, nasally, buccally, vaginally or via an implanted reservoir in dosage formulations containing conventional non-toxic pharmaceutically-acceptable carriers, adjuvants and vehicles. The term parenteral as used herein includes subcutaneous, intravenous, intramuscular, intraperitoneally, intrathecally, intraventricularly, intrasternal and intracranial injection or infusion techniques.

To be effective therapeutically as central nervous system targets the immunophilin-drug complex should readily penetrate the blood-brain barrier when peripherally administered. Compounds of this invention which cannot penetrate the blood-brain barrier can be effetively administered by an intraventricular route.

The pharmaceutical compositions may be in the form of a sterile injectable preparation, for example as a sterile injectable aqueous or oleaginous suspension. This suspension may be formulated according to techniques know in the art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent, for example as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono- or diglycerides. Fatty acids such as oleic acid and its glyceride derivatives find use in the preparation of injectables, olive oil or castor oil, especially in their polyoxyethylated versions. These oil solutions or suspensions may also contain a long-chain alcohol diluent or dispersant.

The compounds may be administered orally in the form of capsules or tablets, for example, or as an aqueous suspension or solution. In the case of tablets for oral use, carriers which are commonly used include lactose and corn starch. Lubricating agents, such as magnesium stearate, are also typically added. For oral

administration in a capsule form, useful diluents include lactose and dried corn starch. When aqueous suspensions are required for oral use, the active ingredient is combined with emulsifying and suspending agents. If desired, certain sweetening and/or flavoring and/or coloring agents may be added.

The compounds of this invention may also be administered in the form of suppositories for rectal administration of the drug. These compositions can be prepared by mixing the drug with a suitable non-irritating excipient which is solid at room temperature but liquid at rectal temperature and therefore will melt in the rectum to release the drug. Such materials include cocoa butter, beeswax and polyethylene glycols.

The compounds of this invention may also be administered optically, especially when the conditions addressed for treatment involve areas or organs readily accessible by topical application, including neurological disorders of the eye, the skin, or the lower intestinal tract. Suitable topical formulations are readily prepared for each of these areas.

For ophthalmic use, the compounds can be formulated as micronized suspensions in isotonic, pH adjusted sterile saline, or, preferably, as solutions is isotonic, pH adjusted sterile saline, either with or without a preservative such as benzylalkonium chloride. Alternatively for the ophthalmic uses the compounds may be formulated in an ointment such as petrolatum.

For application topically to the skin, the compounds can be formulated in a suitable ointment containing the compound suspended or dissolved in, for example, a mixture with one or more of the following: mineral oil, liquid petrolatum, white petrolatum, propylene glycol, polyoxyethylene polyoxypropylene compound, emulsifying wax and water. Alternatively, the compounds can be formulated in a suitable lotion or cream containing the active compound suspended or dissolved in, for example, a mixture of one or more of the following: mineral oil, sorbitan monostearate, polysorbate 60, cetyl esters wax, cetearyl alcohol, 2-octyldodecanol, benzyl alcohol and water.

Topical application for the lower intestinal tract an be effected in a rectal suppository formulation (see above) or in a suitable enema formulation.

Dosage levels on the order of about .1mg to about 10,000 mg. of the active ingredient compound are useful in the treatment of the above conditions, with preferred levels of about 0.1mg to about 1,000 mg. The amount of active ingredient that may be combined with the carrier materials to produce a single dosage form will vary depending upon the host treated and the particular mode of administration.

It is understood, however, that a specific dose level for any particular patient will depend upon a variety of factors including the activity of the specific compound employed, the age, body weight,

general health, sex, diet, time of administration, rate of excretion, drug combination, and the severity of the particular disease being treated and form of administration.

The compounds can be administered with other neurotrophic agents such as neurotrophic growth factor (NGF), glial derived growth factor, brain derived growth factor, ciliary neurotrophic factor, and neurotropin-3. The dosage level of other neurotrophic drugs will depend upon the factors previously stated and the neurotrophic effectiveness of the drug combination.

K_i Test Procedure

Inhibition of the peptidyl-prolyl isomerase (rotamase) activity of the inventive compounds can be evaluated by known methods described in the literature (Harding, M.W. et al. *Nature* 341: 758-760 (1989); Holt et al. *J. Am. Chem. Soc.* 115: 9923-9938). These values are obtained as apparent K_i's and are presented for some of Examples 1-30 in Table I. The *cis-trans* isomerization of an alanine-proline bond in a model substrate, N-succinyl-Ala-Ala-Pro-Phe-*p*-nitroanilide, is monitored spectrophotometrically in a chymotrypsin-coupled assay, which releases *para*-nitroanilide from the *trans* form of the substrate. The inhibition of this reaction caused by the addition of different concentrations of inhibitor is determined, and the data is analyzed as a change in first-order rate constant as

a function of inhibitor concentration to yield the apparent K_i values.

In a plastic cuvette are added 950 mL of ice cold assay buffer (25 mM HEPES, pH 7.8, 100 mM NaCl), 10 mL of FKBP (2.5 mM in 10 mM Tris-Cl pH 7.5, 100 mM NaCl, 1 mM dithiothreitol), 25 mL of chymotrypsin (50 mg/ml in 1 mM HCl) and 10 mL of test compound at various concentrations in dimethyl sulfoxide. The reaction is initiated by the addition of 5 mL of substrate (succinyl-Ala-Phe-Pro-Phe-para-nitroanilide, 5 mg/mL in 2.35 mM LiCl in trifluoroethanol).

The absorbance at 390 nm versus time is monitored for 90 sec using a spectrophotometer and the rate constants are determined from the absorbance versus time data files.

The data for these experiments is presented in Table I.

TABLE I

FKBP ROTAMASE INHIBITION

Example	K_inM
4	42
5	125
6	200
7	65
8	2500
9	160
10	52
24	9000

In mammalian cells, FKBP-12 complexes with the inositol triphosphate receptor (IP_3R) and the ryanodine receptor (RyR). It is believed that the neurotrophic compounds of this invention disassociates FKBP-12 from these complexes causing the calcium channel to become "leaky" (Cameron et al., 1995). Calcium fluxes are involved in neurite extensions so that the IP_3R receptor and the ryanodine receptor might be involved in the neurotrophic effects of drugs. Since the drugs bind to the same site as FKBP-12 as the IP_3R receptor, one could assume that the drugs displace the channels from FKBP-12.

Chick Dorsal Root Ganglion Cultures and Neurite Outgrowth

Dorsal root ganglia were dissected from chick embryos of ten day gestation. Whole ganglion explants were cultured on thin layer Matrigel-coated 12 well plates with Liebovitz L15 plus high glucose media supplemented with 2mM glutamine and 10% fetal calf serum, and also containing 10 μM cytosine β-D arabinofuranoside (Ara C) at 37°C in an environment containing 5% CO_2. Twenty-four hours later, the DRGs were treated with various concentrations of nerve growth factor, immunophilin ligands or combinations of NFG plus drugs. Forty-eight hours after drug treatment, the ganglia were visualized under phase contrast or Hoffman Modulation contrast with a Zeiss Axiovert inverted microscope. Photomicrographs of the explants were made, and neurite outgrowth was quantitated. Neurites longer than the DRG diameter were counted as positive, with total number of neurites quantitated per each experimental condition. Three to four DRGs are cultured per well, and each treatment was performed in duplicate.

The data for these experiments are presented in Table II.

TABLE II

Neurite Outgrowth in Chick DRG

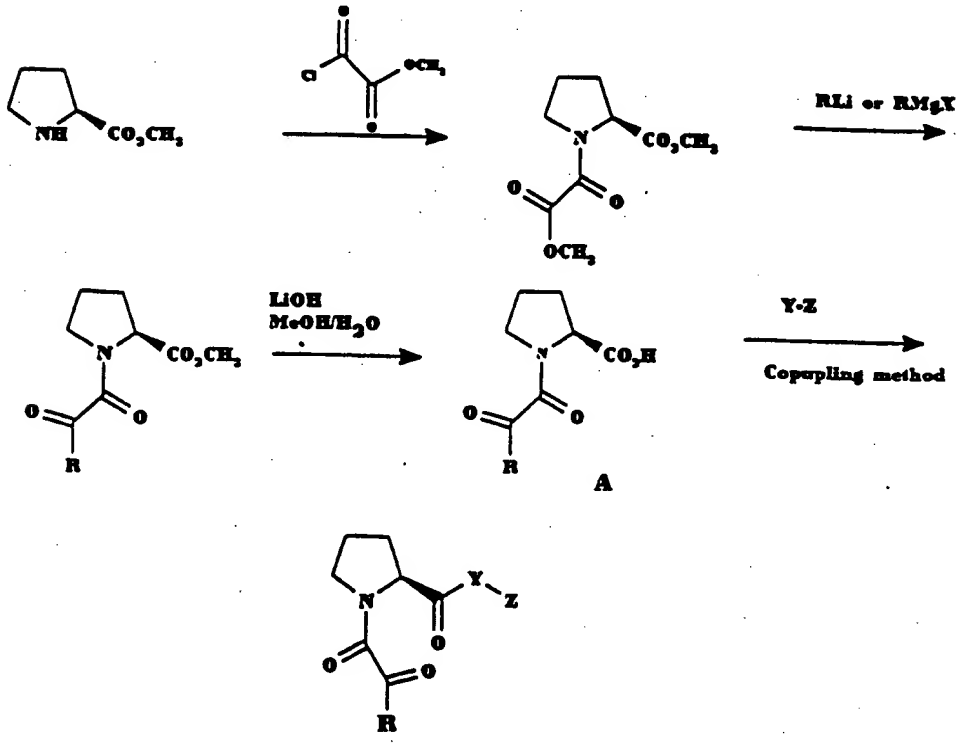
Example	ED_{50} (nM)
4	53
5	105
6	149
7	190
8	850
9	75
10	------
24	------

The following examples are illustrative of preferred embodiments of the invention and are not to be construed as limiting the invention thereto. All polymer molecular weights are mean average molecular weights. All percentages are based on the percent by weight of the final delivery system or formulation prepared unless otherwise indicated and all totals equal 100% by weight.

EXAMPLES

The inventive compounds may be prepared by a variety of synthetic sequences that utilize established chemical transformations. The general pathway to the present compounds is described in Scheme 1. N-glyoxylproline derivatives may be prepared by reacting

L-proline methyl ester with methyl oxalyl chloride as shown in Scheme I. The resulting oxamates may be reacted with a variety of carbon nucleophiles to obtain intermediates compounds. These intermediates are then reacted with a variety of alcohols, amides, or protected amino acid residues to obtain the propyl esters and amides of the invention.



Scheme I

EXAMPLE 1

Synthesis of methyl (2S)-1-(1,2-dioxo-2-methoxyethyl)-2-pyrrolidinecarboxylate.

A solution of L-proline methyl ester hydrochloride (3.08 g; 18.60 mmol) in dry methylene chloride was cooled to 0°C and treated with triethylamine (3.92 g; 38.74 mmol; 2.1 eq). After stirring the formed slurry under a nitrogen atmosphere for 15 min, a solution of methyl oxalyl chloride (3.20 g; 26.12 mmol) in methylene chloride (45 mL) was added dropwise. The resulting mixture was stirred at 0°C for 1.5 hr. After filtering to remove solids, the organic phase was washed with water, dried over $MgSO_4$ and concentrated. The crude residue was purified on a silica gel column, eluting with 50% ethyl acetate in hexane, to obtain 3.52 g (88%) of the product as a reddish oil. Mixture of cis-trans amide rotamers; data for trans rotamer given. ^1H NMR ($CDCl_3$): d 1.93 (dm, 2H); 2.17 (m, 2H); 3.62 (m, 2H); 3.71 (s, 3H); 3.79, 3.84 (s, 3H total); 4.86 (dd, 1H, J = 8.4, 3.3).

EXAMPLE 2

General procedure for the synthesis of pyrrolidinyl alkyl oxamates. Exemplified for methyl (2S)-1-(1,2-dioxo-3,3-dimethylpentyl)-2-pyrrolidinecarboxylate.

A solution of methyl (2S)-1-(1,2-dioxo-2-methoxyethyl)-2-pyrrolidinecarboxylate (2.35 g; 10.90

mmol) in 30 mL of tetrahydrofuran (THF) was cooled to -78°C and treated with 14.2 mL of a 1.0 M solution of 1,1-dimethylpropylmagnesium chloride in THF. After stirring the resulting homogeneous mixture at -78°C for three hours, the mixture was poured into saturated ammonium chloride (100 mL) and extracted into ethyl acetate. The organic phase was washed with water, dried, and concentrated, and the crude material obtained upon removal of the solvent was purified on a silica gel column, eluting with 25% ethyl acetate in hexane, to obtain 2.10 g (75%) of the oxamate as a colorless oil. ^{1}H NMR ($CDCl_3$): d 0.88 (t, 3H); 1.22, 1.26 (s, 3H each); 1.75 (dm, 2H); 1.87-2.10 (m, 3H); 2.23 (m, 1H); 3.54 (m, 2H); 3.75 (s, 3H); 4.52 (dm, 1H, J = 8.4, 3.4).

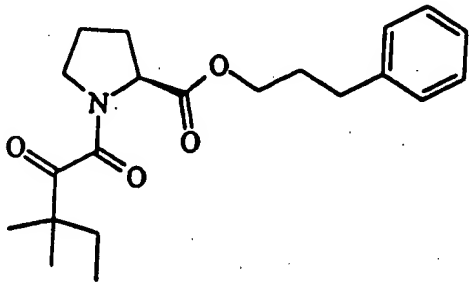
EXAMPLE 3

General procedure for the preparation of pyrrolidine carboxylic acids. Exemplified for (2S)-1-(1,2-dioxo-3,3-dimethylpentyl)-2-pyrrolidinecarboxylic acid.

A mixture of methyl (2S)-1-(1,2-dioxo-3,3-dimethylpentyl)-2-pyrrolidinecarboxylate (2.10 g; 8.23 mmol), 1 N LiOH (15 mL), and methanol (50 mL) was stirred at 0°C for 30 min and at room temperature overnight. The mixture was acidified to pH 1 with 1 N HCl, diluted with water, and extracted into 100 mL of methylene chloride. The organic extract was washed with

brine and concentrated to deliver 1.73 g (87%) of snow-white solid which did not require further purification. ^{1}H NMR ($CDCl_3$): d 0.87 (t, 3H); 1.22, 1.25 (s, 3H each); 1.77 (dm, 2H); 2.02 (m, 2H); 2.17 (m, 1H); 2.25 (m, 1H); 3.53 (dd, 2H, J = 10.4, 7.3); 4.55 (dd, 1H, J = 8.6, 4.1).

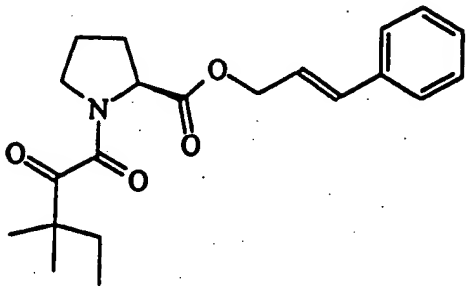
EXAMPLE 4



General procedure for the synthesis of prolyl esters. Exemplified for 3-phenyl-1-propyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate. A mixture of (2S)-1-(1,2-dioxo-3,3-dimethylpentyl)-2-pyrrolidine-carboxylic acid (600 mg; 2.49 mmol), 3-phenyl-1-propanol (508 mg; 3.73 mmol), dicyclohexylcarbodiimide (822 mg; 3.98 mmol), camphorsulphonic acid (190 mg; 0.8 mmol) and 4-dimethylaminopyridine (100 mg; 0.8 mmol) in methylene chloride (20 mL) was stirred overnight under a nitrogen atmosphere. The reaction mixture was filtered through Celite to remove solids and concentrated in vacuo, and the crude material was purified on a flash column (25% ethyl acetate in hexane) to obtain 720 mg (80%) of the

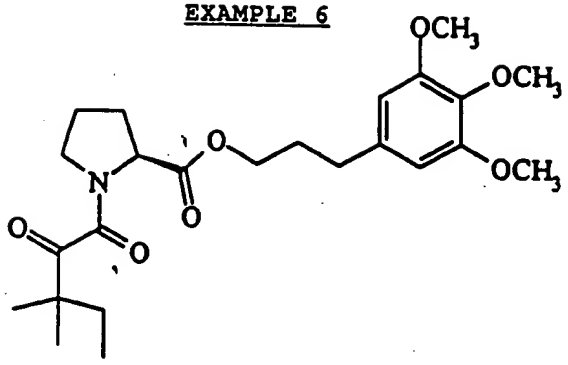
product as a colorless oil. ^{1}H NMR ($CDCl_3$): d 0.84 (t, 3H); 1.19 (s, 3H); 1.23 (s, 3H); 1.70 (dm, 2H); 1.98 (m, 5H); 2.22 (m, 1H); 2.64 (m, 2H); 3.47 (m, 2H); 4.14 (m, 2H); 4.51 (d, 1H); 7.16 (m, 3H); 7.26 (m, 2H).

EXAMPLE 5



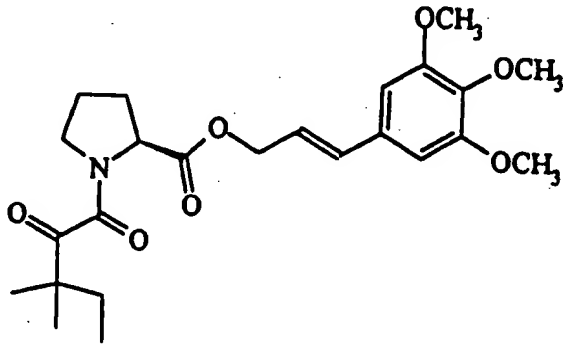
3-Phenyl-1-prop-2-(E)-enyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate, 80%, ^{1}H NMR (360 Mhz, $CDCl_3$): d 0.86 (t, 3H); 1.21 (s, 3H); 1.25 (s, 3H); 1.54-2.10 (m, 5H); 2.10-2.37 (m, 1H); 3.52-3.55 (m, 2H); 4.56 (dd, 1H, J = 3.8, 8.9); 4.78-4.83 (m, 2H); 6.27 (m, 1H); 6.67 (dd, 1H, J = 15.9); 7.13-7.50 (m, 5H). This compound was prepared by the method of Example 3 from (2S)-1-(1,2-dioxo-3,3-dimethylpentyl)-2-pyrrolidine-carboxylic acid.

EXAMPLE 6



3-(3,4,5-Trimethoxyphenyl)-1-propyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate, 61%, ^{1}H NMR ($CDCl_3$): d 0.84 (t, 3H); 1.15 (s, 3H); 1.24 (s, 3H); 1.71 (dm, 2H); 1.98 (m, 5H); 2.24 (m, 1H); 2.63 (m, 2H); 3.51 (t, 2H); 3.79 (s, 3H); 3.83 (s, 3H); 4.14 (m, 2H); 4.52 (m, 1H); 6.36 (s, 2H). This compound was prepared by the method of Example 3 from (2S)-1-(1,2-dioxo-3,3-dimethylpentyl)-2-pyrrolidine-carboxylic acid.

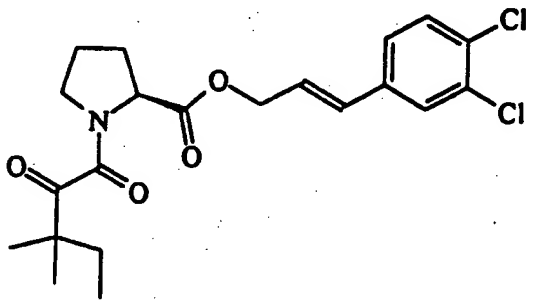
EXAMPLE 7



3-(3,4,5-Trimethoxyphenyl)-1-prop-2-(E)-enyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate, 66%, ^{1}H NMR ($CDCl_3$): d 0.85 (t,

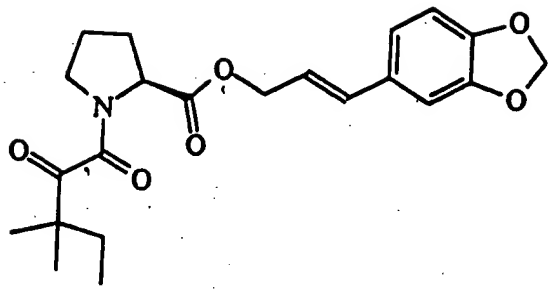
3H); 1.22 (s, 3H); 1.25 (s, 3H); 1.50-2.11 (m, 5H); 2.11-2.40 (m, 1H); 3.55 (m, 2H); 3.85 (s, 3H); 3.88 (s, 6H); 4.56 (dd, 1H); 4.81 (m, 2H); 6.22 (m, 1H); 6.58 (d, 1H, J = 16); 6.63 (s, 2H). This compound was prepared by the method of Example 3 from (2S)-1-(1,2-dioxo-3,3-dimethylpentyl)-2-pyrrolidine-carboxylic acid.

EXAMPLE 8



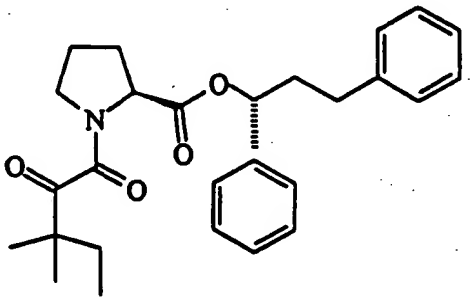
3-,4,5-Dichlorophenyl)-1-prop-2-(E)-enyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate, 70%, ^{1}H NMR ($CDCl_3$): d 0.85 (t, 3H); 1.21 (s, 3H); 1.25 (s, 3H); 1.51-1.87 (m, 2H); 1.87-2.39 (m, 4H); 3.51-3.57 (m, 2H); 4.50-4.61 (dd, 1H, J = 3.4, 8.6); 4.80 (d, 2H, J = 6.0); 6.20-6.34 (m, 1H); 6.50-6.66 (d, 1H, J = 16); 7.13-7.24 (dd, 1H, J = 1.8, 8.3); 7.39 (d, 1H, J = 8.3); 7.47 (s, 1H). This compound was prepared by the method of Example 3 from (2S)-1-(1,2-dioxo-3,3-dimethylpentyl)-2-pyrrolidine-carboxylic acid.

EXAMPLE 9



3-(4,5-Methylenedioxyphenyl)-1-prop-2-(E)-enyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate, 82%, ^{1}H NMR (360 MHz, $CDCl_3$): d 0.86 (t,3H); 1.22 (s, 3H); 1.25 (s, 3H); 1.60-2.10 (m, 5H); 3.36-3.79 (m, 2H); 4.53 (dd, 1H, J = 3.8, 8.6); 4.61-4.89 (m, 2H); 5.96 (s, 2H); 6.10 (m, 1H); 6.57 (dd, 1H, J' = 6.2, 15.8); 6.75 (d, 1H, J = 8.0); 6.83 (dd, 1H, J = 1.3, 8.0); 6.93 (s, 1H). This compound was prepared by the method of Example 3 from (2S)-1-(1,2-dioxo-3,3-dimethylpentyl)-2-pyrrolidine-carboxylic acid.

EXAMPLE 10

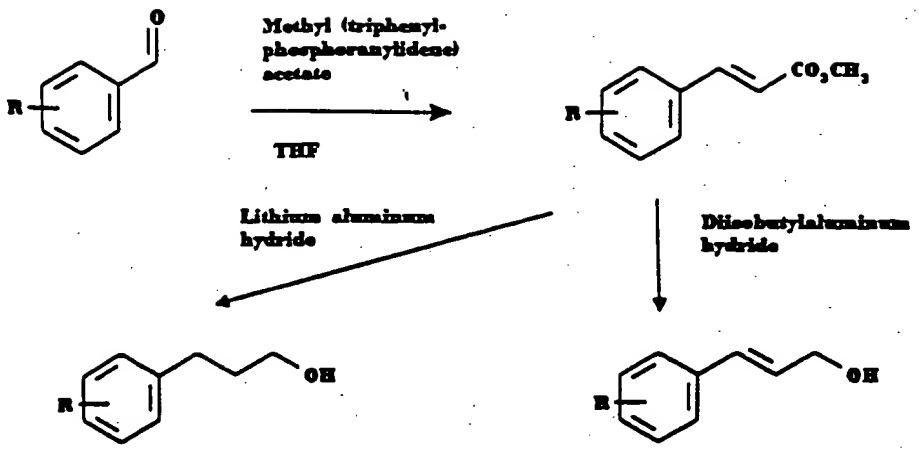


(1R)-1,3-Diphenyl-1-propyl (2S)-1-(3,3-dimethyl-

1,2-dioxopentyl)-2-pyrrolidinecarboxylate, 90%, ^{1}H NMR (360 MHz, $CDCl_3$): d 0.85 (t, 3H); 1.20 (s, 3H); 1.23 (s, 3H); 1.49-2.39 (m, 7H); 2.46-2.86 (m, 2H); 3.25-3.80 (m, 2H); 4.42-4.82 (m, 1H); 5.82 (td, 1H, J = 1.8, 6.7); 7.05-7.21 (m, 3H); 7.21-7.46 (m, 7H). This compound was prepared by the method of Example 3 from (2S)-1-(1,2-dioxo-3,3-dimethylpentyl)-2-pyrrolidine-carboxylic acid.

EXAMPLE 11

The requisite substituted alcohols may be prepared by a number of methods known to those skilled in the art of organic synthesis. As described in Scheme II, substituted benzaldehydes may be homologated to phenyl propanols by reaction with methyl (triphenylphosphoranylidene)- acetate to provide a variety of *trans*-cinnamates; these latter may be reduced to the saturated alcohols by reaction with excess lithium aluminum hydride, or sequentially by reduction of the double bond by catalytic hydrogenation and reduction of the saturated ester by appropriate reducing agents. Alternatively, the *trans*-cinnamates may be reduced to (E)-allylic alcohols by the use of diisobutylaluminum hydride.



Scheme II

Longer chain alcohols may be prepared by homologation of benzylic and higher aldehydes. Alternatively, these aldehydes may be prepared by conversion of the corresponding phenylacetic and higher acids, and phenethyl and higher alcohols.

EXAMPLE 12

General procedure for the synthesis of acrylic esters, exemplified for methyl (3,3,5-trimethoxy)-*trans*-cinnamate.

A solution of 3,4,5-trimethoxybenzaldehyde (5.0 g; 25.48 mmol) and methyl (triphenyl-phosphoranylidene)acetate (10.0 g; 29.91 mmol) in tetrahydrofuran (250 mL) was refluxed overnight. After cooling, the reaction mixture was diluted with 200 mL of ethyl acetate and washed with 2 x 200 mL of water, dried, and concentrated in vacuo. The crude residue was chromatographed on a silica gel column, eluting with

25% ethyl acetate in hexane, to obtain 5.63 g (88%) of the cinnamate as a white crystalline solid, ^{1}H NMR (300 Mhz; $CDCl_3$): d 3.78 (s, 3H); 3.85 (s, 6H); 6.32 (d, 1H, J = 16); 6.72 (s, 2H); 7.59 (d, 1H, J = 16).

EXAMPLE 13

Methyl (4,5-dichloro)-*trans*-cinnamate, 80%, ^{1}H NMR (300 Mhz; $CDCl_3$): d 3.79 (s, 3H); 6.40 (d, 1H, J = 16.8); 7.32 (dd, 1H, J = 1.5, 8.1); 7.44 (d, 1H, J = 8.1); 7.56 (d, 1H, J = 16); 7.58 (s, 1H). This compound was prepared by the method of Example 11 from 3,4,5-trimethoxybenzaldehyde.

EXAMPLE 14

Methyl (4,5-methylenedioxy)-*trans*-cinnamate, 74%, ^{1}H NMR (360 Mhz; $CDCl_3$): d 3.79 (s, 3H); 6.01 (s, 2H); 6.26 (d, 1H, J = 16); 6.81 (d, 1H, J = 7.9); 7.00 (d, 1H, J = 8.2); 7.03 (s, 1H); 7.60 (d, 1H, J = 16). This compound was prepared by the method of Example 11 from 3,4,5-trimethoxybenzaldehyde.

EXAMPLE 15

Methyl (2-cyclohexyl)-(E)-acrylate, 80%, ^{1}H NMR (360 Mhz; $CDCl_3$): d 1.12-1.43 (m, 5H); 1.52-1.87 (m, 5H); 2.12 (m, 1H); 3.71 (s, 3H); 5.77 (dd, 1H, J = 1.2, 15.8); 6.92 (dd, 1H, J = 6.8, 15.8). This compound was prepared by the method of Example 11 from 3,4,5-trimethoxybenzaldehyde.

EXAMPLE 16

General procedure for the synthesis of saturated alcohols from acrylic esters. Exemplified for (3,4,5-

trimethoxy) phenylpropanol.

A solution of methyl (3,3,5-trimethoxy)-*trans*-cinnamate (1.81 g; 7.17 mmol) in tetrahydrofuran (30 mL) was added in a dropwise manner to a solution of lithium aluminum hydride (14 mmol) in THF (35 mL), with stirring and under an argon atmosphere. After the addition was complete, the mixture was heated to 75°C for 4 hours. After cooling, it was quenched by the careful addition of 15 mL of 2N NaOH followed by 50 mL of water. The resulting mixture was filtered through Celite to remove solids, and the filter cake was washed with ethyl acetate. The combined organic fractions were washed with water, dried, concentrated in vacuo, and purified on a silica gel column, eluting with ethyl acetate to obtain 0.86 g (53%) of the alcohol as a clear oil, ^{1}H NMR (300 Mhz; $CDCl_3$): d 1.23 (br, 1H); 1.87 (m, 2H); 2.61 (t, 2H, J = 7.1); 3.66 (t, 2H); 3.80 (s, 3H); 3.83 (s, 6H); 6.40 (s, 2H).

EXAMPLE 17

General procedure for the synthesis of trans-allylic alcohols from acrylic esters. Exemplified for (3,4,5-trimethoxy)phenylprop-2-(E)-enol.

A solution of methyl (3,3,5-trimethoxy)-*trans*-cinnamate (1.35 g; 5.35 mmol) in toluene (25 mL) was cooled to -10°C and treated with a solution of diisobutylaluminum hydride in toluene (11.25 mL of a 1.0 M solution; 11.25 mmol). The reaction mixture was stirred for 3 hrs at 0°C and then quenched with 3 mL of

methanol followed by 1 N HCl until the pH was 1. The reaction mixture was extracted into ethyl acetate and the organic phase was washed with water, dried and concentrated. Purification on a silica gel column eluting with 25% ethyl acetate in hexane furnished 0.96 g (80%) of a thick oil, ^{1}H NMR (360 Mhz; $CDCl_3$): d 3.85 (s, 3H); 3.87 (s, 6H); 4.32 (d, 2H, J = 5.6); 6.29 (dt, 1H, J = 15.8, 5.7), 6.54 (d, 1H, J = 15.8); 6.61 (s, 2H).

EXAMPLE 18

(4,5-dichloro)phenylprop-2-(E)-enol, 89%, ^{1}H NMR (360 Mhz; $CDCl_3$): d 1.55 (s, 1H); 4.34 (d, 2H, J = 4.4); 6.36 (dt, 1H, J = 15.9, 5.3); 6.54 (d, 1H, J = 15.9); 7.20 (dd, 1H, J = 8.3, 1.7); 7.38 (d, 1H, J = 8.3); 7.45 (d, 1H, J = 1.6). This compound was prepared by the method of Example 16 from (3,4,5-trimethoxy)-trans-cinnamate.

EXAMPLE 19

(4,5-methylenedioxy)phenylprop-2-(E)-enol, 80%, ^{1}H NMR (360 Mhz; $CDCl_3$): d 1.59 (br, 1H); 4.29 (br, 2H); 5.96 (s, 2H); 6.20 (dt, 1H, J = 15.8, 5.9); 6.52 (d, 1H, J = 15.8); 6.76 (d, 1H, J = 8.0); 6.82 (dd, 1H, J = 8.0, 1.2); 6.93 (d, 1H, J = 1.2). This compound was prepared by the method of Example 16 from (3,4,5-trimethoxy)-trans-cinnamate.

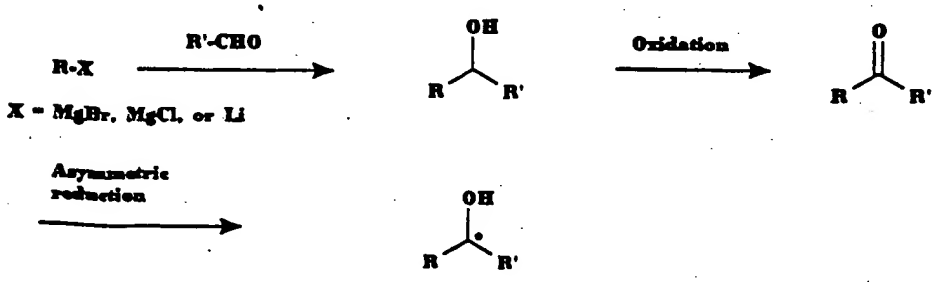
EXAMPLE 20

Phenylprop-2-(E)-enol, 85%, ^{1}H NMR (360 Mhz; $CDCl_3$): d 1.72 (br, 1H); 4.31 (d, 2H, J = 5.7); 6.36

(dt, 1H, J = 15.9, 5.7); 6.61 (d, 1H, J = 15.9); 7.02-7.55 (m, 5H). This compound was prepared by the method of Example 16 from (3,4,5-tri-methoxy)-trans-cinnamate.

EXAMPLE 21

Alcohols containing a substituent at the 1-position of the side chain may be conveniently prepared by addition of appropriate nucleophiles to aldehydes, as described in Scheme III. In cases where optically active substituted alcohols are desired, the racemic alcohols may be oxidized to prochiral ketones and subjected to asymmetric reduction by one of several methods well known to those skilled in the art.



Scheme III

EXAMPLE 22

General procedure for the preparation of 1-substituted alkanols, exemplified for the synthesis of 1,3-diphenylpropanol.

A solution of 2-(bromoethyl)benzene (17.45 g; 94.3 mmol) in 50 mL of dry diethyl ether was added dropwise, under a nitrogen atmosphere, to a stirred slurry of magnesium turnings (2.50 g; 102.8 mmol) in 50 mL of ether. The mixture was initially heated with a heat gun until reflux had become self-sustaining. After the addition was complete, the mixture was heated externally for 30 min to maintain reflux. A solution of 10.01 g (94.3 mmol) of benzaldehyde in 20 mL of ether was then added dropwise, and reflux was continued for 30 min. After cooling, the reaction mixture was poured into 150 mL of saturated ammonium chloride and extracted into ethyl acetate. The crude material obtained upon removal of the solvent was purified on a flash column, eluting with 5% ethyl acetate/hexane to 20% ethyl acetate, to obtain 13.73 g (69%) of the alkanol as a light yellow oil, ^{1}H NMR (360 Mhz; $CDCl_3$): d 1.93-2.30 (m, 3H); 2.70-2.90 (m, 2H); 4.72 (br, 1H); 7.19-7.27 (m, 3H); 7.27-7.36 (m, 3H); 7.36-7.47 (m, 4H).

EXAMPLE 23

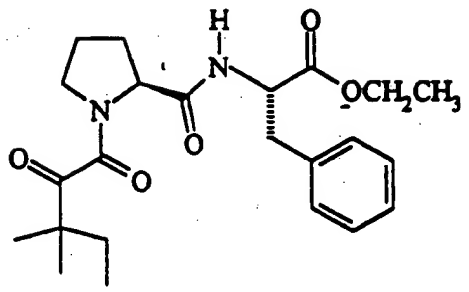
General procedure for conversion of racemic 1-substituted alkanols to optically active 1-substituted alkanols via prochiral ketones. Exemplified for (1R)-1,3-diphenyl-1-propanol.

A solution of racemic 1,3-diphenyl-1-propanol (1.26 g; 5.94 mmol) was dissolved in 10 mL of acetone, and Jones reagent was added until persistence of the

orange color. After stirring for 30 min, the reaction was quenched by adding 2 mL of 2-propanol. The solvent was decanted away from the precipitated solids, which were washed with ethyl acetate. The combined organic fractions were washed with 2 x 20 mL of water, dried and concentrated. The crude product was filtered through a plug of silica gel, eluting with 25% ethyl acetate/hexane, to obtain 1.07 g (86%) of 1,3-diphenylpropanone as a white crystalline solid, ^{1}H NMR (360 Mhz; $CDCl_3$): d 3.09 (t, 2H, J = 8.1); 3.33 (t, 2H, J = 8.1); 7.29 (m, 5H); 7.49 (m, 3H); 7.98 (m, 2H).

A solution of 1,3-diphenylpropanone (1.07 g; 5.09 mmol) in tetrahydrofuran (10 mL) was cooled to -23°C and treated with an asymmetric reducing agent, (+)-B-chlorodiisopinocampheyl-borane (1.80 g; 5.60 mmol) in 20 mL THF, and the resulting solution was allowed to stand overnight at -23°C. After evaporating to dryness, the residue was treated with ether (65 mL) and diethanolamine (1.0 g) and stirred for 3 hrs. The mixture was then filtered to remove solids and concentrated, and the residue was purified using gradient elution (5% ethyl acetate/hexane to 10% ethyl acetate) on a silica gel column to obtain 660 mg (61%) of (1R)-1,3-diphenyl-1-propanol as a crystalline white solid, ^{1}H NMR (360 Mhz; $CDCl_3$): d 1.95-2.15 (m, 3H); 2.59-2.78 (m, 2H); 4.65 (dd, 1H, J = 5.4, 7.8); 7.14-7.35 (m, 10H).

EXAMPLE 24



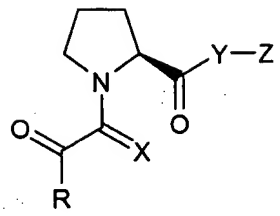
General procedure for the synthesis of prolyl dipeptides, exemplified for 1-[1-(3,3-dimethyl-1,2-dioxopentyl)-L-proline]-L-phenylalanine ethyl ester.

A mixture of (2S)-1-(1,2-dioxo-3,3-dimethylpentyl)-2-pyrrolidinecarboxylic acid (1.17 g; 4.85 mmol), L-phenylalanine ethyl ester hydrochloride (1.23 g; 5.33 mmol), dicyclohexylcarbo- diimide (1.10 g; 5.33 mmol) and 4-dimethylaminopyridine (60 mg (4.85 mmol) in methylene chloride (25 mL) was treated with triethylamine (1 mL; 726 mg; 7.17 mmol) and stirred overnight. The mixture was filtered through Celite to remove solids and concentrated, and the crude material from removal of the solvent was purified on a silica gel column eluting with 30% ethyl acetate/hexane to obtain 2.02 g of 1-[1-(3,3-dimethyl-1,2-dioxopentyl)-L-proline]-L-phenylalanine ethyl ester, 100%, ^{1}H NMR (360 MHz, $CDCl_3$): d 0.87 (t, 3H); 1.16-1.28 (m, 9H); 1.58-1.91 (m, 5H); 2.33 (m, 1H); 3.07-3.20 (m, 2H); 3.38-3.41 (m, 2H); 4.11-4.18 (m, 4H); 4.55 (d, 1H, J = 6.5); 4.78-4.80 (m, 1H); 7.15 (br d, 1H); 7.19 (m, 5H).

The invention being thus described, it will be obvious that the same may be varied in many ways. Such variations are not to be regarded as a departure from the spirit and scope of the invention and all such modification are intended to be included within the scope of the following claims.

What is claimed is:

1. A neurotrophic compound of the formula:



where

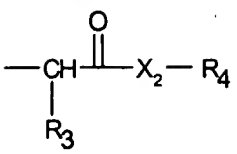
R_1 is a C_1-C_9 straight or branched chain alkyl or alkenyl group optionally substituted with C_3-C_8 cycloalkyl, C_3 or C_5 cycloalkyl, C_5-C_7 cycloalkenyl, or Ar_1, where said alkyl, alkenyl, cycloalkyl or cycloalkenyl groups may be optionally substituted with C_1-C_4 alkyl, C_1-C_4 alkenyl, or hydroxy, and where Ar_1 is selected from the group consisting of 1-napthyl, 2-napthyl, 2-indolyl, 3-indolyl, 2-furyl, 3-furyl, 2-thienyl, 3-thienyl, 2-, 3-, or 4-pyridyl, or phenyl, having one to three substituents which are independently selected from the

group consisting of hydrogen, halo, hydroxyl, nitro, trifluoromethyl, C_1-C_6 straight or branched alkyl or alkenyl, C_1-C_4 alkoxy or C_1-C_4 alkenyloxy, phenoxy, benzyloxy, and amino;

X is oxygen, sulfur, methylene (CH_2), or H_2;

Y is oxygen or NR_2, where R_2 is hydrogen or C_1-C_6 alkyl; and

Z is a C2-C6 straight or branched chain alkyl or alkenyl, wherein the alkyl chain is substituted in one or more positions with Ar_1 as defined above, C_3-C_8 cycloalkyl, cycloalkyl connected by a C_1-C_6 straight or unbranched alkyl or alkenyl chain, or Ar_2 where Ar_2 is selected from the group consisting of 2-indolyl, 3-indolyl, 2-furyl, 3-furyl, 2-thiazolyl, 2-thienyl, 3-thienyl, 2-, 3-, or 4-pyridyl, or phenyl, having one to three substituents which are independently selected from the group consisting of hydrogen, halo, hydroxyl, nitro, trifluoromethyl, C_1-C_6 straight or branched alkyl or alkenyl, C_1-C_4 alkoxy or C_1-C_4 alkenyloxy, phenoxy, benzyloxy, and amino;

Z may also be  the fragment:

where

R_3 is selected from the group consisiting of straight or branched alkyl C_1-C_8 optionally substituted with C_3-C_8 cycloalkyl, or Ar_1 as defined above;

X_2 is O or NR_5, where R_5 is selected from the group consisting of hydrogen, C_1-C_6 straight or branched alkyl and alkenyl;

R_4 is selected from the group consisting of phenyl, benzyl, C_1-C_5 straight or branched alkyl or alkenyl, and C_1-C_5 straight or branched alkyl or alkenyl substituted with phenyl; or pharmaceutically acceptable salts or hydrates thereof.

2. The neurotrophic compound of claim 1, which has an affinity for FKBP-type immunophilins.

3. The neurotrophic compound of claim 2, where the FKBP-type immunophilin is FKBP-12.

4. The neurotrophic compound of claim 1, capable of inhibiting rotamase activity.

5. The neurotrophic compound of claim 1, where Z and R_1 are lipophilic groups.

6. The neurotrophic compound according to claim 1 that is selected from the group consisting of

3-phenyl-1-propyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate,

3-phenyl-1-prop-2-(E)-enyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate,

3-(3,4,5-trimethoxyphenyl)-1-propyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate,

3-(3,4,5-trimethoxyphenyl)-1-prop-2-(E)-enyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidine carboxylate,

3-(4,5-methylenedioxyphenyl)-1-propyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate,

3-(4,5-methylenedioxyphenyl)-1-prop-2-(E)-enyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate,

3-cyclohexyl-1-propyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate,

3-cyclohexyl-1-prop-2-(E)-enyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate,

(1R)-1,3-diphenyl-1-propyl (2S)-1-(3,3-dimethyl-1,2-

dioxopentyl)-2-pyrrolidinecarboxylate,

3-phenyl-1-propyl (2S)-1-(1,2-dioxo-2-[2-furanyl])ethyl-2-pyrrolidinecarboxylate,

3-phenyl-1-propyl (2S)-1-(1,2-dioxo-2-[2-thienyl])ethyl-2-pyrrolidinecarboxylate,

3-phenyl-1-propyl (2S)-1-(1,2-dioxo-2-[2-thiazolyl])ethyl-2-pyrrolidinecarboxylate,

3-phenyl-1-propyl (2S)-1-(1,2-dioxo-2-phenyl)ethyl-2-pyrrolidinecarboxylate,

3-(2,5-dimethoxyphenyl)-1-propyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate,

3-(2,5-dimethoxyphenyl)-1-prop-2-(E)-enyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidine- carboxylate,

2-(3,4,5-trimethoxyphenyl)-1-ethyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate,

3-(3-Pyridyl)-1-propyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate,

3-(2-Pyridyl)-1-propyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate,

3-(4-Pyridyl)-1-propyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate,

3-phenyl-1-propyl (2S)-1-(2-cyclohexyl-1,2-dioxoethyl)-2-pyrrolidinecarboxylate,

3-phenyl-1-propyl (2S)-1-(2-*tert*-butyl-1,2-dioxoethyl)-2-pyrrolidinecarboxylate,

3-phenyl-1-propyl (2S)-1-(2-cyclohexylethyl-1,2-dioxoethyl)-2-pyrrolidinecarboxylate,

3-(3-pyridyl)-1-propyl (2S)-1-(2-cyclohexylethyl-1,2-dioxoethyl)-2-pyrrolidinecarboxylate,

3-(3-pyridyl)-1-propyl (2S)-1-(2-*tert*-butyl-1,2-dioxoethyl)-2-pyrrolidinecarboxylate,

3,3-diphenyl-1-propyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate,

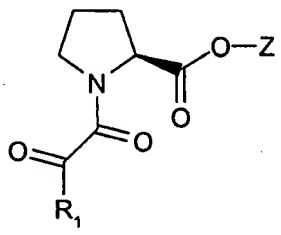
3-(3-pyridyl)-1-propyl (2S)-1-(2-cyclohexyl-1,2-dioxoethyl)-2-pyrrolidinecarboxylate,

3-(3-Pyridyl)-1-propyl (2S)-N-([2-thienyl] glyoxyl)pyrrolidinecarboxylate,

3,3-Diphenyl-1-propyl (2*S*)-1-(3,3-dimethyl-1,2-dioxobutyl)-2-pyrrolidinecarboxylate,

3,3-Diphenyl-1-propyl (2*S*)-1-cyclohexylglyoxyl-2-pyrrolidinecarboxylate,

3,3-Diphenyl-1-propyl (2*S*)-1-(2-thienyl)glyoxyl-2-pyrrolidinecarboxylate.

7. A pharmaceutical composition comprising a neurotrophically effective amount of  the compound of claim 1 and a pharmaceutically acceptable carrier.

8. A method of stimulating growth of damaged peripheral nerves, which comprises:

administering to damaged peripheral nerves the neurotrophic compound of claim 1 in sufficient amounts to stimulate the growth of said nerves.

9. A neurotrophic compound of the formula:

where

R_1 is a C_1-C_9 straight or branched chain alkyl or alkenyl group optionally substituted with C_3-C_8 cycloalkyl, C_3 or C_5 cycloalkyl, C_5-C_7 cycloalkenyl, or Ar_1, where said alkyl, alkenyl, cycloalkyl or cycloalkenyl groups may be optionally substituted with C_1-C_4 alkyl, C_1-C_4 alkenyl, or hydroxy, and where Ar_1 is selected from the group consisting of 1-napthyl, 2-napthyl, 2-indolyl, 3-indolyl, 2-furyl, 3-furyl, 2-thiazolyl, 2-thienyl, 3-thienyl, 2-, 3-, or 4-pyridyl, or phenyl, having one to three substituents which are independently selected from the group consisting of hydrogen, halo, hydroxyl, nitro, trifluoromethyl, C_1-C_6 straight or branched alkyl or alkenyl, C_1-C_4 alkoxy or C_1-C_4 alkenyloxy, phenoxy, benzyloxy, and amino;

Z is a C_2-C_6 straight or branched chain alkyl or alkenyl, wherein the alkyl chain is substituted in one or more positions with Ar_1 as defined above, C_3-C_8 cycloalkyl,

the group consisting of 2-indolyl, 3-indolyl, 2-furyl, 3-furyl, 2-thiazolyl, 2-thienyl, 3-thienyl, 2-, 3-, or 4-pyridyl, or phenyl, having one to three substituents which are independently selected from the group consisting of hydrogen, halo, hydroxyl, nitro, trifluoromethyl, C_1-C_6 straight or branched alkyl or alkenyl, C_1-C_4 alkoxy or C_1-C_4 alkenyloxy, phenoxy, benzyloxy, and amino; or pharmaceutically acceptable salts or hydrates thereof.

10. The neurotrophic compound of claim 9, wherein R_1 is selected from the group consisting of C_1-C_9 straight or branched chain alkyl, cyclohexyl, cyclopentyl, 2-furanyl, 2- thienyl, 2-thiazolyl, and 4-hydroxybutyl.

11. The neurotrophic compound of claim 9 having an affinity for FKBP-type immunophilins.

12. The neurotrophic compounds of claim 11, where the FKBP-type immunophilin is FKBP-12.

13. The neurotrophic compound of claim 9, capable of inhibiting rotamase activity.

14. The neurotrophic compound of claim 9, where Z and R_1 are liphophilic groups.

15. A pharmaceutical composition comprising a neurotrophically effective amount of the compound of claim 9 and a pharmaceutically acceptable carrier.

16. A neurotrophic compound having an affinity for FKBP-type immunophilins wherein the immunophilin exhibits rotamase activity and the neurotrophic compound inhibits the rotamase activity of the immunophilin.

17. The neurotrophic compound of claim 16, wherein the FKBP-type immunophilin is FKBP-12.

18. A method of treating a neurological disorder in an animal

comprising administering a therapeutically effective amount of a compound having an affinity for FKBP-type immunophilins wherein the immunophilin exhibits rotamase activity and the neurotrophic compound inhibits the rotamase activity of the immunophilin.

19. The method of claim 18, wherein the FKBP-type immunophilin is FKBP-12.

20. The method of claim 18, wherein the neurological disorder is selected from the group consisting of peripheral neuropathies, and neurological pathologies related to neurodegeneration.

21. The method of claim 18, wherein the neurological disorder is Alzheimer's disease.

22. The method of claim 18, wherein the neurological disorder is Parkinson's disease.

23. The method of claim 18, wherein the neurological disorder is amyotrophic lateral sclerosis.

24. A method of promoting neuronal regeneration and growth in mammals, comprising administering to a subject an effective amount of a neurotrophic compound having an affinity for FKBP-type immunophilins wherein the immunophilin exhibits rotamase activity and the neurotrophic compound inhibits the rotamase activity of the immunophilin.

25. The method of claim 24, wherein the FKBP-type immunophilin is FKBP-12.

26. A method of preventing neurodegeneration in an animal comprising administering an effective amount of a compound having an affinity for FKBP-type immunophilins wherein the immunophilin exhibits rotamase activity and the neurotrophic compound inhibits the rotamase activity of the immunophilin.

27. The method of claim 26, wherein the FKBP-type immunophilin is FKBP-12.

ABSTRACT OF THE DISCLOSURE

This invention relates to neurotrophic compounds having an affinity for FKBP-type immunophilins, their preparation and use as inhibitors of the enzyme activity associated with immunophilin proteins, and particularly inhibitors of peptidyl-prolyl isomerase or rotamase enzyme activity.

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(Rev. 8-95)

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JOSEPH P. STEINER, FINKSBURG, MD; GREGORY S. HAMILTON, CATONSVILLE, MD.

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TITLE

ROTAMAS ENZYME ACTIVITY INHIBITORS

PRELIMINARY CLASS: 514

(see reverse)

Attorney Docket No.: 22903

Title:

ROTAMASE ENZYME ACTIVITY INHIBITORS

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1. **Field of the Invention**

This invention relates to the method of using neurotrophic FKBP inhibitor compounds having an affinity for FKBP-type immunophilins as inhibitors of the enzyme activity associated with immunophilin proteins, and particularly inhibitors of peptidyl-prolyl isomerase or rotamase enzyme activity.

2. **Description of the Prior Art**

The term immunophilin refers to a number of proteins that serve as receptors for the principal immunosuppressant drugs, cyclosporin A (CsA), FK506, and rapamycin. Known classes of immunophilins are cyclophilins, and FK506 binding proteins, such as FKBP. Cyclosporin A binds to cyclophilin while FK506 and rapamycin bind to FKBP. These immunophilin-drug complexes interface with a variety of intracellular signal transduction systems, especially in the immune system and the nervous system.

Immunophilins are known to have peptidyl-prolyl isomerase (PPIase) or rotamase enzyme activity. It has been determined that rotamase activity has a role in the catalyzation of the interconversion of the cis and trans isomer of immunophilin proteins.

Immunophilins were originally discovered and studied in immune tissue. It was initially postulated by those

skilled in the art that inhibition of the immunophilins rotamase activity leads to the inhibition of T-cell proliferation, thereby causing the immunosuppressive action exhibited by immunosuppressive drugs such as cyclosporin A, FK506, and rapamycin. Further study has shown that the inhibition of rotamase activity, in and of itself, is not sufficient for immunosuppressant activity. Instead immunosuppression appears to stem from the formulation of a complex of immunosuppressant drugs and immunophilins. It has been shown that the immunophilin-drug complexes interact with ternary protein targets as their mode of action. In the case of FKBP-FK506 and FKBP-CsA, the drug-immunophilin complexes bind to the enzyme calcineurin, inhibiting T-cell receptor signalling leading to T-cell proliferation. Similarly, the complex of rapamycin and FKBP interacts with the RAFT1/FRAP protein and inhibits signalling from the IL-2 receptor.

Immunophilins have been found to be present at high concentrations in the central nervous system. Immunophilins are enriched 10-50 times more in the central nervous system than in the immune system. Within neural tissues, immunophilins appear to influence nitric oxide synthesis, neurotransmitter release, and neuronal process extension.

FK506 also augments the phosphorylation of growth-associated protein-43 (GAP43). GAP43 is involved in

neuronal process extension and its phosphorylation appears to augment this activity. Accordingly, the effects of FK506 rapamycin and cyclosporin in neuronal process extension have been examined using PC12 cells. PC12 cells are a continuous line of neuronal-like cells which extend neurites when stimulated by nerve growth factor (NGF).

Surprisingly, it has been found that picomolar concentrations of an immunosuppressant such as FK506 and rapamycin stimulate neurite out growth in PC12 cells and sensory neurons, namely dorsal root ganglion cells (DRGs). In whole animal experiments, FK506 has been shown to stimulate nerve regeneration following facial nerve injury and results in functional recovery in animals with sciatic nerve lesions.

More particularly, it has been found that drugs with a high affinity for FKBP are potent rotamase inhibitors and exhibit excellent neurotrophic effects. Snyder et al., "Immunophilins and the Nervous System", *Nature Medicine*, Volume 1, No. 1, January 1995, 32-37. These findings suggest the use of immunosuppressants in treating various peripheral neuropathies and enhancing neuronal regrowth in the central nervous system (CNS). Studies have demonstrated that neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis (ALS) may

occur due to the loss, or decreased availability, of a neurotrophic substance specific for a particular population of neurons affected in the disorder.

Several neurotrophic factors effecting specific neuronal populations in the central nervous system have been identified. For example, it has been hypothesized that Alzheimer's disease results from a decrease or loss of nerve growth factor (NGF). It has thus been proposed to treat SDAT patients with exogenous nerve growth factor or other neurotrophic proteins such as brain derived growth factor, glial derived growth factor, ciliary neurotrophic factor, and neurotropin-3 to increase the survival of degenerating neuronal populations.

Clinical application of these proteins in various neurological disease states is hampered by difficulties in the delivery and bioavailability of large proteins to nervous system targets. By contrast, immunosuppressant drugs with neurotrophic activity are relatively small and display excellent bioavailability and specificity. However, when administered chronically, immunosuppressants exhibit a number of potentially serious side effects including nephrotoxicity, such as impairment of glomerular filtration and irreversible interstitial fibrosis (Kopp et al., 1991, *J. Am. Soc. Nephrol.* 1:162); neurological deficits, such as involuntary tremors, or non-specific cerebral angina such

as non-localized headaches (De Groen et al., 1987, *N. Engl. J. Med.* 317:861); and vascular hypertension with complications resulting therefrom (Kahan et al., 1989 *N. Engl. J. Med.* 321: 1725).

The present invention provides non-immunosuppressive FKBP inhibitor compounds containing small molecule FKBP rotamase inhibitors which are extremely potent in augmenting neurite outgrowth, and for promoting neuronal growth, and regeneration in various neuropathological situations where neuronal repair can be facilitated including peripheral nerve damage by physical injury or disease state such as diabetes, physical damage to the central nervous system (spinal cord and brain), brain damage associated with stroke, and for the treatment of neurological disorders relating to neurodegeneration, including Parkinson's disease, Alzheimer's disease, and amyotrophic lateral sclerosis.

SUMMARY OF THE INVENTION

This invention relates to the method of using neurotrophic FKBP inhibitor compounds having an affinity for FKBP-type immunophilins as inhibitors of the enzyme activity associated with immunophilin proteins, and particularly inhibitors of peptidyl-prolyl isomerase or rotamase enzyme activity.

A preferred embodiment of this invention is a method

of treating a neurological activity in an animal, comprising: administering to an animal an effective amount of a FKBP inhibitor having an affinity for FKBP-type immunophilins to stimulate growth of damaged peripheral nerves or to promote neuronal regeneration, wherein the FKBP-type immunophilin exhibits rotamase activity and the pipecolic acid derivative inhibits said rotamase activity of the immunophilin.

Another preferred embodiment of this invention is a method of treating a neurological disorder in an animal, comprising: administering to an animal an effective amount of a FKBP inhibitor having an affinity for FKBP-type immunophilins in combination with an effective amount of a neurotrophic factor selected from the group consisting of neurotrophic growth factor, brain derived growth factor, glial derived growth factor, cilial neurotrophic factor, and neurotropin-3, to stimulate growth of damaged peripheral nerves or to promote neuronal regeneration, wherein the FKBP-type immunophilin exhibits rotamase activity and the pipecolic acid derivative inhibits said rotamase activity of the immunophilin.

Another preferred embodiment of this invention is a method of stimulating growth of damaged peripheral nerves, comprising: administering to damaged peripheral nerves an effective amount of an FKBP inhibitor compound

having an affinity for FKBP-type immunophilins to stimulate or promote growth of the damaged peripheral nerves, wherein the FKBP-type immunophilins exhibit rotamase activity and the pipecolic acid derivative inhibits said rotamase activity of the immunophilin.

Another preferred embodiment of this invention is a method of stimulating growth of damaged peripheral nerves, comprising: administering to damaged peripheral nerves an effective amount of an FKBP inhibitor compound having an affinity for FKBP-type immunophilins to stimulate growth of damaged peripheral nerves, wherein the FKBP-type immunophilin exhibit rotamase activity and the pipecolic acid derivative inhibits said rotamase activity of the immunophilin.

Another preferred embodiment of this invention is a method for promoting neuronal regeneration and growth in animals, comprising: administering to an animal an effective amount of an FKBP inhibitor compound having an affinity for FKBP-type immunophilins to promote neuronal regeneration, wherein the FKBP-type immunophilins exhibit rotamase activity and the pipecolic acid derivative inhibits said rotamase activity of the immunophilin. Yet another preferred embodiment of this invention is a method for preventing neurodegeneration in an animal, comprising: administering to an animal an effective amount of an FKBP inhibitor having an affinity

for FKBP-type immunophilins to prevent neurodegeneration, wherein the FKBP-type immunophilin exhibits rotamase activity and the pipecolic acid derivative inhibits said rotamase activity of the immunophilin.

DETAILED DESCRIPTION OF THE INVENTION

The novel neurotrophic FKBP inhibitor compounds of this invention have an affinity for the FK506 binding proteins such as FKBP-12. When the neurotrophic compounds of the invention are bound to FKBP, they have been found to inhibit the prolyl-peptidyl cis-trans isomerase activity, or rotamase activity of the binding protein and unexpectedly stimulate neurite growth.

The compounds of the present invention can be used in the form of salts derived from inorganic or organic acids and bases. Included among such acid salts are the following: acetate, adipate, alginate, aspartate, benzoate, benzenesulfonate, bisulfate butyrate, citrate, camphorate, camphorsulfonate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, fumarate, glucoheptanoate, glycerophosphate, hemissulfate heptanoate, hexanoate, hydrochloride, hydrobromide, hydroiodide, 2-hydroxyethanesulfonate, lactate, maleate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, oxalate, pamoate, pectinate, propionate, succinate, tartrate, thiocyanate, tosylate and undecanoate. Base

salts include ammonium salts, alkali metal salts such as sodium and potassium salts, alkaline earth metal salts such as calcium and magnesium salts, salt with organic bases such as dicyclohexylamine salts, N-methyl-D-glucamine, and salts with amino acids such as arginine, lysine, and so forth. Also, the basic nitrogen-containing groups can be quarternized with such agents as lower alkyl halides, such as methyl, ethyl, propyl, and butyl chlorides, bromides and iodides; dialkyl sulfates such as dimethyl, diethyl, dibutyl and diamyl sulfates; long chain halides such as decyl, lauryl, myristyl and stearyl chlorides, bromides and iodides; aralkyl halides like benzyl and phenethyl bromides; and others. Water or oil-soluble or dispersible products are thereby obtained.

The neurotrophic compounds of this invention can be periodically administered to a patient undergoing treatment for neurological disorders or for other reasons in which it is desirable to stimulate neuronal regeneration and growth, such as in various peripheral neuropathic and neurological disorders relating to neurodegeneration. The compounds of this invention can also be administered to mammals other than humans for treatment of various mammalian neurological disorders.

The novel compounds of the present invention are potent inhibitors of rotamase activity and possess an

excellent degree of neurotrophic activity. This activity is useful in the stimulation of damaged neurons, the promotion of neuronal regeneration, the prevention of neurodegeneration, and in the treatment of several neurological disorders known to be associated with neuronal degeneration and peripheral neuropathies. The neurological disorders that may be treated include but are not limited to: trigeminal neuralgia, glossopharyngeal neuralgia, Bell's Palsy, myasthenia gravis, muscular dystrophy, amyotrophic lateral sclerosis, progressive muscular atrophy, progressive bulbar inherited muscular atrophy, herniated, ruptured or prolapsed invertabrae disk syndromes, cervical spondylosis, plexus disorders, thoracic outlet destruction syndromes, peripheral neuropathic such as those caused by lead, dapsone, ticks, porphyria, or Gullain-Barré syndrome, Alzheimer's disease, and Parkinson's disease.

For these purposes the compounds of the present invention may be administered orally, parenterally, by inhalation spray, topically, rectally, nasally, buccally, vaginally or via an implanted reservoir in dosage formulations containing conventional non-toxic pharmaceutically-acceptable carriers, adjuvants and vehicles. The term parenteral as used herein includes subcutaneous, intravenous, intramuscular,

intraperitoneally, intrathecally, intraventricularly, intrasternal and intracranial injection or infusion techniques.

To be effective therapeutically as central nervous system targets, the immunophilin-drug complex should readily penetrate the blood-brain barrier when peripherally administered. Compounds of this invention which cannot penetrate the blood-brain barrier can be effectively administered by an intraventricular route.

The pharmaceutical compositions may be in the form of a sterile injectable preparation, for example as a sterile injectable aqueous or oleaginous suspension. This suspension may be formulated according to techniques know in the art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent, for example as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono- or diglycerides. Fatty acids such as oleic acid and its glyceride derivatives find use in the preparation of injectables,

olive oil or castor oil, especially in their polyoxyethylated versions. These oil solutions or suspensions may also contain a long-chain alcohol diluent or dispersant.

The compounds may be administered orally in the form of capsules or tablets, for example, or as an aqueous suspension or solution. In the case of tablets for oral use, carriers which are commonly used include lactose and corn starch. Lubricating agents, such as magnesium stearate, are also typically added. For oral administration in a capsule form, useful diluents include lactose and dried corn starch. When aqueous suspensions are required for oral use, the active ingredient is combined with emulsifying and suspending agents. If desired, certain sweetening and/or flavoring and/or coloring agents may be added.

The compounds of this invention may also be administered in the form of suppositories for rectal administration of the drug. These compositions can be prepared by mixing the drug with a suitable non-irritating excipient which is solid at room temperature but liquid at rectal temperature and therefore will melt in the rectum to release the drug. Such materials include cocoa butter, beeswax and polyethylene glycols.

The compounds of this invention may also be administered optically, especially when the conditions

addressed for treatment involve areas or organs readily accessible by topical application, including neurological disorders of the eye, the skin, or the lower intestinal tract. Suitable topical formulations are readily prepared for each of these areas.

For ophthalmic use, the compounds can be formulated as micronized suspensions in isotonic, pH adjusted sterile saline, or, preferably, as solutions is isotonic, pH adjusted sterile saline, either with or without a preservative such as benzylalkonium chloride. Alternatively for the ophthalmic uses the compounds may be formulated in an ointment such as petrolatum.

For application topically to the skin, the compounds can be formulated in a suitable ointment containing the compound suspended or dissolved in, for example, a mixture with one or more of the following: mineral oil, liquid petrolatum, white petrolatum, propylene glycol, polyoxyethylene polyoxypropylene compound, emulsifying wax and water. Alternatively, the compounds can be formulated in a suitable lotion or cream containing the active compound suspended or dissolved in, for example, a mixture of one or more of the following: mineral oil, sorbitan monostearate, polysorbate 60, cetyl esters wax, cetearyl alcohol, 2-octyldodecanol, benzyl alcohol and water.

Topical application for the lower intestinal tract

an be effected in a rectal suppository formulation (see above) or in a suitable enema formulation.

Dosage levels on the order of about 0.1 mg to about 10,000 mg of the active ingredient compound are useful in the treatment of the above conditions, with preferred levels of about 0.1 mg to about 1,000 mg. The amount of active ingredient that may be combined with the carrier materials to produce a single dosage form will vary depending upon the host treated and the particular mode of administration.

It is understood, however, that a specific dose level for any particular patient will depend upon a variety of factors including the activity of the specific compound employed, the age, body weight, general health, sex, diet, time of administration, rate of excretion, drug combination, and the severity of the particular disease being treated and form of administration.

The compounds can be administered with other neurotrophic agents such as neurotrophic growth factor (NGF), glial derived growth factor, brain derived growth factor, ciliary neurotrophic factor, and neurotropin-3. The dosage level of other neurotrophic drugs will depend upon the factors previously stated and the neurotrophic effectiveness of the drug combination.

Methods and Procedures

Nerve Extension Elicited in Chick Dorsal Root Ganglia by Non-Immunosuppressive Ligands of Immunophilins

In previous studies, it has been observed that neurotrophic effects of immunosuppressant drugs in explants of rat dorsal root ganglia with significant augmentation in nerve outgrowth has occurred with FK506 concentrations as low as 1 picomolar (Lyons et. al., 1994). In the rat ganglia neurotrophic effects were observed with FK506 even in the absence of NGF. In the present work explants of chick dorsal root ganglia have been used, which are easier to employ in studies of nerve outgrowth. In the absence of added NGF, we have observed minimal effects of immunophilin ligand drugs. The chick cells are more sensitive to NGF than PC-12 cells so that we employ 0.1 ng/ml NGF to produce minimal neurite outgrowth and to demonstrate neurotrophic actions of immunophilin ligands (Fig. 5).

The maximal increase in the number of processes, their length and branching is quite similar at maximally effective contractions of the immunophilin ligands and of NGF (100 ng/ml). With progressively increasing concentrations of the various drugs, one observes a larger number of processes, more extensive branching and a greater length of individual processes.

We evaluated the potencies of drugs in binding to FKBP-12 by examining inhibition of peptidyl prolyl-isomerase activity and inhibition of ^{3}H-FK506 binding to recombinant FKBP-12 (Table 1). There is a striking parallel between their potencies in stimulating neurite outgrowth and inhibiting rotamase activity.

The very close correlation between the potencies of drugs in binding to immunophilins, inhibiting their rotamase activity and stimulating neurite outgrowth implies that inhibition of rotamase activity is responsible for neurotrophic effects of the drugs. The extraordinarily high potency of the drugs in stimulating neurite outgrowth and in binding to immunophilins makes it most unlikely that any other target could account for the neurotrophic effects.

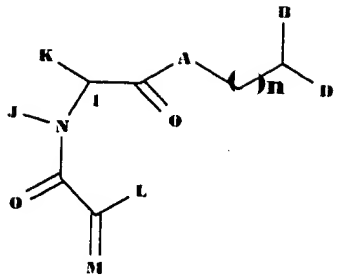
Because of the extraordinary potency of the drugs and the close correlation between rotamase inhibition and neurotrophic actions, we conclude that rotamase inhibition is likely involved in neurotrophic effects. A number of proteins have been reported as substrates for the rotamase activity of immunophilins including collagen (Steinmann et. al., 1991) and transferring (Lodish and King, 1991). Recently highly purified preparations of ryanodine receptor and the IP-3 receptor, prominent intracellular calcium channels, have been reported to exist in a complex with FKBP-12. Dissociation of FKBP-12

from these complexes causes the calcium channels to become "leaky" (Cameron et. al., 1995). Calcium fluxes are involved in neurite extension so that the IP-3 receptor and the ryanodine receptor might be involved in the neurotrophic effects of drugs. Since the drugs bind to the same site on FKBP-12 as the IP-3 receptor or the ryanodine receptor, one would have to postulate that the drugs displace the channels from FKBP-12. No interaction between these calcium channels in cyclophilin has been reported so that this model would not explain the neurotrophic actions of cyclosporin A.

The neurotrophic actions of the drugs studied here are exerted at extremely low concentrations indicating potencies comparable to those of neurotrophic proteins such as brain derived growth factor, neurotropin-3 and neurotrophic growth factor.

The following examples are illustrative of preferred embodiments of the invention and are not to be construed as limiting the invention thereto. All polymer molecular weights are mean average molecular weights. All percentages are based on the percent by weight of the final delivery system or formulation prepared unless otherwise indicated and all totals equal 100% by weight.

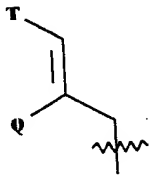
Illustrative generic FKBP inhibitor compounds which can be used for the purposes of this invention include:



and pharmaceutically acceptable salts thereof,

wherein A is CH_2, O, NH, or N-(C_1-C_4 alkyl);

wherein B and D are independently Ar, (C5-C7)-cycloalkyl substituted (C1-C6)-straight or branched alkyl or alkenyl, (C5-C7)-cycloalkenyl substituted (C1-C6)-straight or branched alkyl or alkenyl, or Ar substituted (C1-C6)-straight or branched alkyl or alkenyl, wherein in each case, one or two carbon atoms of the straight or branched alkyl or alkenyl groups may be substituted with 1-2 heteroatoms selected from the group consisting of oxygen, sulfur, SO and SO_2 in chemically reasonable substitution patterns, or



wherein Q is hydrogen, (C1-C6)-straight or branched alkyl or (C1-C6)-straight or branched alkenyl;

wherein T is Ar or substituted 5-7 membered cycloalkyl with substituents at positions 3 and 4 which

are independently selected from the group consisting of hydrogen, hydroxyl, O-(C1-C4)-alkyl or O-(C1-C4)-alkenyl and carbonyl;

wherein Ar is selected from the group consisting of 1-napthyl, 2-napthyl, 2-furyl, 3-furyl, 2-thienyl, 3-thienyl, 2-pyridyl, 3-pyridyl, 4-pyridyl and phenyl, monocyclic and bicyclic heterocyclic ring systems with individual ring sizes being 5 or 6 which may contain in either or both rings a total of 1-4 heteroatoms independently selected from oxygen, nitrogen and sulfur; wherein Ar may contain one to three substituents which are independently selected from the group consisting of hydrogen, halo, hydroxyl, hydroxymethyl, nitro, CF3, trifluoromethoxy, (C1-C6)-straight or branched alkyl or (C1-C6)-straight or branched alkenyl, O-(C1-C4)-straight or branched alkyl or O-(C1-C4)-straight or branched alkenyl, O-benzyl, O-phenyl, amino, 1,2-methylenedioxy, carbonyl and phenyl;

wherein L is either hydrogen or U; M is either oxygen or CH-U, provided that if L is hydrogen, then M is CH-U, or if M is oxygen then L is U;

wherein U is hydrogen, O-(C1-C4)-straight or branched alkyl or O-(C1-C4)-straight or branched alkenyl, (C1-C6)-straight or branched alkyl or (C1-C6)-straight or branched alkenyl, (C5-C7)-cycloalkyl, (C5-C7)-cycloalkenyl substituted with (C1-C4)-straight or

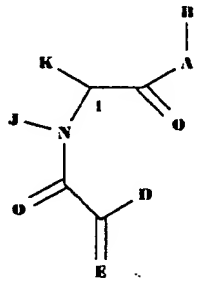
branched alkyl or (C1-C4)-straight or branched alkenyl, [(C1-C4)-alkyl or (C1-C4)-alkenyl]-Ar or Ar (Ar as described above);

wherein J is hydrogen or C1 or C2 alkyl or benzyl; K is (C1-C4)-straight or branched alkyl, benzyl or cyclohexylethyl; or wherein J and K may be taken together to form a 5-7 membered heterocyclic ring which may contain an oxygen (O), sulfur (S), SO or SO_2 substituted therein; and

wherein n is 0-3.

The stereochemistry at position 1 (Formula I) is (R) or (S), with (S) preferred. The stereochemistry at position 2 is (R) or (S).

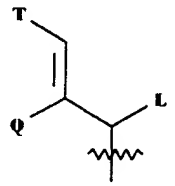
Illustrative preferred FKBP inhibitor compounds which can be used for the purposes of this invention are described in U.S. Patent No. 5,330,993, the contents of which is incorporated herein by reference. Exemplary compounds include those having the formula:



and pharmaceutically acceptable salts thereof,

wherein A is O, NH, or N-(C1-C4 alkyl);

wherein B is hydrogen, CHL-Ar, (C1-C6)-straight or branched alkyl, (C1-C6)-straight or branched alkenyl, (C5-C7)-cycloalkyl, (C5-C7)-cycloalkenyl or Ar substituted (C1-C6)-alkyl or alkenyl, or



wherein L and Q are independently hydrogen, (C1-C6)-straight or branched alkyl or (C1-C6)-straight or branched alkenyl;

wherein T is Ar or substituted cyclohexyl with substituents at positions 3 and 4 which are independently selected from the group consisting of hydrogen, hydroxyl, O-(C1-C4)-alkyl or O-(C1-C4)-alkenyl and carbonyl;

wherein Ar is selected from the group consisting of 1-napthyl, 2-napthyl, 2-furyl, 3-furyl, 2-thienyl, 2-pyridyl, 3-pyridyl, 4-pyridyl and phenyl having one to three substituents which are independently selected from the group consisting of hydrogen, halo, hydroxyl, nitro,

CF3, (C1-C6)-straight or branched alkyl or (C1-C6)-straight or branched alkenyl, O-(C1-C4)-straight or branched alkyl or O-(C1-C4)-straight or branched alkenyl, O-benzyl, O-phenyl, amino and phenyl;

wherein D is either hydrogen or U; E is either oxygen or CH-U, provided that if D is hydrogen, then E is CH-U, or if E is oxygen then D is U;

wherein U is hydrogen, O-(C1-C4)-straight or branched alkyl or O-(C1-C4)-straight or branched alkenyl, (C1-C6)-straight or branched alkyl or (C1-C6)-straight or branched alkenyl, (C5-C7)-cycloalkyl, (C5-C7)-cycloalkenyl substituted with (C1-C4)-straight or branched alkyl or (C1-C4)-straight or branched alkenyl, 2-indolyl, 3-indolyl, [(C1-C4)-alkyl or (C1-C4)-alkenyl]-Ar or Ar (Ar as described above);

wherein J is hydrogen or C1 or C2 alkyl or benzyl; K is (C1-C4)-straight or branched alkyl, benzyl or cyclohexylethyl; or wherein J and K may be taken together to form a 5-7 membered heterocyclic ring which may contain an oxygen (O), sulfur (S), SO or SO2 substituted therein.

The stereochemistry at position 1 (Formula I) is (R) or (S), with (S) preferred.

K_i Test Procedure

Inhibition of the peptidyl-prolyl isomerase (rotamase) activity of the compounds used herein can be evaluated by known methods described in the literature (Harding, M.W. et al. *Nature* 341: 758-760 (1989); Holt et al. *J. Am. Chem. Soc.* 115: 9923-9938). These values are obtained as apparent $_K$'s and are presented for various compounds in Table I. The *cis-trans* isomerization of an alanine-proline bond in a model substrate, N-succinyl-Ala-Ala-Pro-Phe-*p*-nitroanilide, is monitored spectrophotometrically in a chymotrypsin-coupled assay, well known to those skilled in the art, which releases *para*-nitroanilide from the *trans* form of the substrate. The inhibition of this reaction caused by the addition of different concentrations of inhibitor is determined, and the data is analyzed as a change in first-order rate constant as a function of inhibitor concentration to yield the apparent $_K$ values.

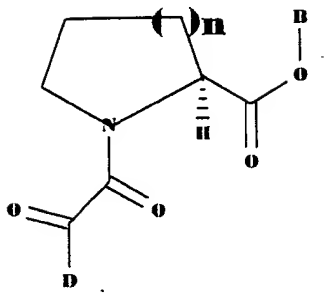
In a plastic cuvette are added 950 mL of ice cold assay buffer (25 mM HEPES, pH 7.8, 100 mM NaCl), 10 mL of FKBP (2.5 mM in 10 mM Tris-Cl pH 7.5, 100 mM NaCl, 1 mM dithiothreitol), 25 mL of chymotrypsin (50 mg/ml in 1 mM HCl) and 10 mL of test compound at various concentrations in dimethyl sulfoxide. The reaction is initiated by the addition of 5 mL of substrate (succinyl-Ala-Phe-Pro-Phe-para-nitroanilide, 5 mg/mL in 2.35 mM

LiCl in trifluoroethanol).

The absorbance at 390 nm versus time is monitored for 90 sec using a spectrophotometer and the rate constants are determined from the absorbance versus time data files.

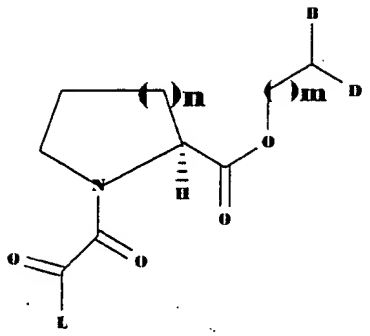
The data for these experiments is presented in Tables I and II.

TABLE I



No.	B	D	n	K
1	Benzyl	Phenyl	2	1.5 μM
2	3-Phenylpropyl	Phenyl	2	
3	4-(4-Methoxy-phenyl)butyl	Phenyl	2	
4	4-Phenylbutyl	Phenyl	2	0.35 μM
5	Phenethyl	Phenyl	2	1.1 μM
6	4-Cyclohexyl-butyl	Phenyl	2	0.4 μM
7	Benzyl	Methoxy	2	80 μM
8	4-Cyclohexyl-butyl	Methoxy	2	6 μM
9	3-Cyclohexyl-propyl	Methoxy	2	20 μM
10	3-Cyclopentyl-propyl	Methoxy	2	35 μM
11	Benzyl	2-Furyl	2	3 μM
12	4-Cyclohexyl-butyl	3,4,5-Trimethoxy-phenyl	2	0.04 μM
13	3-Phenoxy-benzyl	3,4,5-Trimethoxy-phenyl	2	0.018 μM
14	4-Phenylbutyl	3,4,5-Trimethoxy-phenyl	2	0.019 μM
15	3-(3-Indolyl) propyl	3,4,5-Trimethoxy-phenyl	2	0.017 μM
16	4-(4-Methoxy-phenyl)butyl	3,4,5-Trimethoxy-phenyl	2	0.013 μM

TABLE II



No.	n	m	B	D	L
1	2	0	3-Phenylpropyl	3-(3-Pyridyl) propyl	Phenyl
2	2	0	3-Phenylpropyl	3-(2-Pyridyl) propyl	Phenyl
3	2	0	3-Phenylpropyl	2-(4-Methoxy-phenyl)ethyl	Phenyl
4	2	0	3-Phenylpropyl	3-Phenylpropyl	Phenyl
5	2	0	3-Phenylpropyl	3-Phenylpropyl	3,4,5-Trimeth-oxyphenyl
6	2	0	3-Phenylpropyl	2-(3-Pyridyl)	3,4,5-Trimeth-oxyphenyl
7	2	0	3-Phenylpropyl	3-(2-Pyridyl)	3,4,5-Trimeth-oxyphenyl
8	2	0	3-Phenylpropyl	3-(4-Methoxy-phenyl)propyl	3,4,5-Trimeth-oxyphenyl
9	2	0	3-Phenylpropyl	3-(3-Pyridyl) propyl	3-Iso-propoxy-phenyl

Chick Dorsal Root Ganglion Cultures and Neurite Outgrowth

Dorsal root ganglia were dissected from chick embryos of ten day gestation. Whole ganglion explants were cultured on thin layer Matrigel-coated 12 well plates with Liebovitz L15 plus high glucose media supplemented with 2mM glutamine and 10% fetal calf serum, and also containing 10 μM cytosine β-D arabinofuranoside (Ara C) at 37°C in an environment containing 5% CO_2.

Twenty-four hours later, the DRGs were treated with various concentrations of nerve growth factor, immunophilin ligands or combinations of NFG plus drugs. Forty-eight hours after drug treatment, the ganglia were visualized under phase contrast or Hoffman Modulation contrast with a Zeiss Axiovert inverted microscope. Photomicrographs of the explants were made, and neurite outgrowth was quantitated. Neurites longer than the DRG diameter were counted as positive, with total number of neurites quantitated per each experimental condition. Three to four DRGs are cultured per well, and each treatment was performed in duplicate.

The data for these experiments are presented in Table III.

Table III

Neurite Outgrowth in Chick DRG

Example	Neurotrophic Potency
1	++
2	
3	
4	+++
5	++

6	+++
7	+
8	++
9	+
10	+
11	++
12	+++
13	+++
14	+++
15	+++
16	+++

Table IV

Biological Results

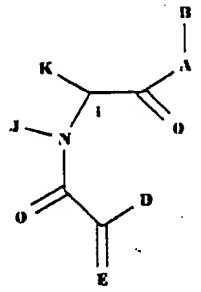
Compound	K, nM	Neurite Outgrowth
1	56	+++
2	50	+++
3	270	++
4	---	---
5	1.0	++++
6	3.0	++++
7	1.0	++++
8	3.0	++++
9	2.0	++++

The invention being thus described, it will be obvious that the same may be varied in many ways. Such variations are not to be regarded as a departure from the spirit and scope of the invention and all such modifications are intended to be included within the scope of the following claims.

What is claimed is:

1. A method of treating a neurological activity in an animal, comprising:

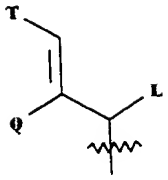
administering to an animal an effective amount of a pipecolic acid derivative represented by the formula



and pharmaceutically acceptable salts thereof,

wherein A is CH_2, O, NH, or N-(C1-C4 alkyl);

wherein B and D are independently Ar, (C5-C7)-cycloalkyl substituted (C1-C6)-straight or branched alkyl or alkenyl, (C5-C7)-cycloalkenyl substituted (C1-C6)-straight or branched alkyl or alkenyl, or Ar substituted (C1-C6)-straight or branched alkyl or alkenyl, wherein in each case, one or two carbon atoms of the straight or branched alkyl or alkenyl groups may be substituted with 1-2 heteroatoms selected from the group consisting of oxygen, sulfur, SO and SO_2 in chemically reasonable substitution patterns, or



wherein Q is hydrogen, (C1-C6)-straight or branched alkyl or (C1-C6)-straight or branched alkenyl;

wherein T is Ar or substituted 5-7 membered cycloalkyl with substituents at positions 3 and 4 which are independently selected from the group consisting of hydrogen, hydroxyl, O-(C1-C4)-alkyl or O-(C1-C4)-alkenyl and carbonyl;

wherein Ar is selected from the group consisting of 1-napthyl, 2-napthyl, 2-furyl, 3-furyl, 2-thienyl, 3-thienyl, 2-pyridyl, 3-pyridyl, 4-pyridyl and phenyl, monocyclic and bicyclic heterocyclic ring systems with individual ring sizes being 5 or 6 which may contain in either or both rings a total of 1-4 heteroatoms independently selected from oxygen, nitrogen and sulfur; wherein Ar may contain one to three substituents which are independently selected from the group consisting of hydrogen, halo, hydroxyl, hydroxymethyl, nitro, CF3, trifluoromethoxy, (C1-C6)-straight or branched alkyl or (C1-C6)-straight or branched alkenyl, O-(C1-C4)-straight

or branched alkyl or 0-(C1-C4)-straight or branched alkenyl, 0-benzyl, 0-phenyl, amino, 1, 2-methylenedioxy, carbonyl and phenyl;

wherein L is either hydrogen or U; M is either oxygen or CH-U, provided that if L is hydrogen, then M is CH-U, or if M is oxygen then L is U;

wherein U is hydrogen, 0-(C1-C4)-straight or branched alkyl or 0-(C1-C4)-straight or branched alkenyl, (C1-C6)-straight or branched alkyl or (C1-C6)-straight or branched alkenyl, (C5-C7)-cycloalkyl, (C5-C7)-cycloalkenyl substituted with (C1-C4)-straight or branched alkyl or (C1-C4)-straight or branched alkenyl [(C1-C4)-alkyl or (C1-C4)-alkenyl]-Ar or Ar (Ar as described above);

wherein J is hydrogen or C1 or C2 alkyl or benzyl; K is (C1-C4)-straight or branched alkyl, benzyl or cyclohexylmethyl; or wherein J and K may be taken together to form a 5-7 membered heterocyclic ring which may contain an oxygen (O), sulfur (S), SO or SO_2 substituted therein;

wherein n is 0-3; and

wherein said pipecolic acid derivative has an affinity for FKBP-type immunophilins, said administering stimulates growth of damaged peripheral nerves or promotes neuronal regeneration, the FKBP-type immunophilin exhibits rotamase activity, and the

pipecolic acid derivative inhibits said rotamase activity of the immunophilin.

2. The method of claim 1, wherein the neuronal activity is selected from the group consisting of stimulation of damaged neurons, promotion of neuronal regeneration, prevention of neurodegeneration and treatment of neurological disorders.

3. The method of claim 2, wherein the neurological disorder is selected from the group consisting of peripheral neuropathies cause by physical injury or disease state, physical damage to the brain, physical damage to the spinal cord, stroke associated with brain damage, and neurological disorders relating to neurodegeneration.

4. The method of claim 3, wherein the neurological disorder is selected from the group consisting of Alzheimer's Disease, Parkinson's Disease, and amyotrophic lateral sclerosis.

5. The method of claim 1, wherein the pipecolic acid derivative compound is immunosuppressive or non-immunosuppressive.

6. A method of treating a neurological activity in an animal, comprising:

administering to an animal an effective amount of a pipecolic acid derivative according to claim 1 having an affinity for FKBP-type immunophilins in combination with an effective amount of a neurotrophic factor selected from the group consisting of neurotrophic growth factor, brain derived growth factor, glial derived growth factor, cilial neurotrophic factor, and neurotropin-3, to stimulate growth of damaged peripheral nerves or to promote neuronal regeneration, wherein the FKBP-type immunophilin exhibits rotamase activity and the pipecolic acid derivative inhibits said rotamase activity of the immunophilin.

7. The method of claim 6, wherein the neuronal activity is selected from the group consisting of stimulation of damaged neurons, promotion of neuronal regeneration, prevention of neurodegeneration and treatment of neurological disorders.

8. The method of claim 7, wherein the neurological disorder is selected from the group consisting of peripheral neuropathies caused by physical injury or disease state, physical damage to the brain, physical damage to the spinal cord, and neurological disorders relating to neurodegeneration.

9. The method of claim 6, wherein the neurological disorder is selected from the group consisting of Alzheimer's Disease, Parkinson's Disease, and amyotrophic lateral sclerosis.

10. The method of claim 6, wherein the pipecolic acid derivative compound is immunosuppressive or non-immunosuppressive.

11. A method of stimulating growth of damaged peripheral nerves, comprising;

administering to damaged peripheral nerves an effective amount of a pipecolic acid derivative compound according to claim 1 having an affinity for FKBP-type immunophilins to stimulate or promote growth of the damaged peripheral nerves, wherein the FKBP-type immunophilins exhibit rotamase activity and the pipecolic acid derivative

inhibits said rotamase activity of the immunophilin.

12. The method of claim 11, further comprising administering a neurotrophic factor to stimulate or promote growth of the damaged peripheral nerves selected from the group consisting of neurotrophic growth factor, brain derived growth factor, glial derived growth factor, cilial neurotrophic factor, and neurotropin-3.

13. The method of claim 11, wherein the pipecolic acid derivative is immunosuppressive or non-immunosuppressive.

14. A method for promoting neuronal regeneration and growth in animals, comprising:

administering to an animal an effective amount of a pipecolic acid derivative compound according to claim 1 having an affinity for FKBP-type immunophilins to promote neuronal regeneration, wherein the FKBP-type immunophilins exhibit rotamase activity and the pipecolic acid derivative inhibits said rotamase activity of the immunophilin.

15. The method of claim 14, further comprising administering an effective amount of a neurotrophic factor to promote neuronal regeneration selected from the

group consisting of neurotrophic growth factor, brain derived growth factor, glial derived growth factor, and neurotropin-3.

16. The method of claim 14, wherein the pipecolic acid derivative compound is immunosuppressive or non-immunosuppressive.

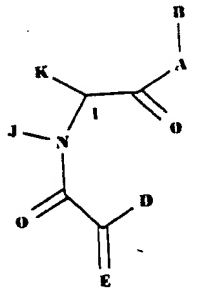
17. A method for preventing neurodegeneration in an animal, comprising:

administering to an animal an effective amount of a pipecolic acid derivative according to claim 1 having an affinity for FKBP-type immunophilins to prevent neurodegeneration, wherein the FKBP-type immunophilin exhibits rotamase activity and the pipecolic acid derivative inhibits said rotamase activity of the immunophilin.

18. The method of claim 17, further comprising administering an effective amount of a neurotrophic factor to prevent neurodegeneration selected from the group consisting of neurotropic growth factor, brain derived growth factor, glial derived growth factor, cilial neurotropic factor, and neurotropin-3.

19. The method of claim 17, wherein the pipecolic acid derivative compound is immunosuppressive or non-immunosuppressive.

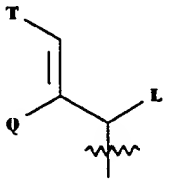
20. The method of treating a neurological activity according to claim 1 represented by the formula:



and pharmaceutically acceptable salts thereof,

wherein A is O, NH, or N-(C1-C4 alkyl);

wherein B is hydrogen, CHL-Ar, (C1-C6)-straight or branched alkyl, (C1-C6)-straight or branched alkenyl, (C5-C7)-cycloalkyl, (C5-C7)-cycloalkenyl or Ar substituted (C1-C6)-alkyl or alkenyl, or



wherein L and Q are independently hydrogen, (C1-C6)-straight or branched alkyl or (C1-C6)-straight or branched alkenyl;

wherein T is Ar or substituted cyclohexyl with substituents at positions 3 and 4 which are independently selected from the group consisting of hydrogen, hydroxyl, O-(C1-C4)-alkyl or O-(C1-C4)-alkenyl and carbonyl;

wherein Ar is selected from the group consisting of 1-napthyl, 2-napthyl, 2-furyl, 3-furyl, 2-thienyl, 2-pyridyl, 3-pyridyl, 4-pyridyl and phenyl having one to three substituents which are independently selected from the group consisting of hydrogen, halo, hydroxyl, nitro, CF3, (C1-C6)-straight or branched alkyl or (C1-C6)-straight or branched alkenyl, O-(C1-C4)-straight or branched alkyl or O-(C1-C4)-straight or branched alkenyl, O-benzyl, O-phenyl, amino and phenyl.

wherein D is either hydrogen or U; E is either oxygen or CH-U, provided that if D is hydrogen, then E is CH-U, or if E is oxygen then D is U;

wherein U is hydrogen, O-(C1-C4)-straight or branched alkyl or O-(C1-C4)-straight or branched alkenyl, (C1-C6)-straight or branched alkyl or (C1-C6)-straight or branched alkenyl, (C5-C7)-cycloalkyl, (C5-C7)-cycloalkenyl substituted with (C1-C4)-straight or branched alkyl or (C1-C4)-straight or branched alkenyl, 2-indolyl, 3-indolyl, [(C1-C4)-alkyl or (C1-C4)1alkenyl]-

Ar or Ar (Ar as described above);

wherein J is hydrogen or C1 or C2 alkyl or benzyl; K is (C1-C4)-straight or branched alkyl, benzyl or cyclohexylethyl; or wherein J and K may be taken together to form a 5-7 membered heterocyclic ring which may contain an oxygen (O), sulfur (S), SO or SO2 substituted therein.

ABSTRACT OF THE DISCLOSURE

This invention relates to the method of using specially formulated neurotrophic pipecolic acid derivative compounds having an affinity for FKBP-type immunophilins as inhibitors of the enzyme activity associated with immunophilin proteins, and particularly inhibitors of peptidyl-prolyl isomerase or rotamase enzyme activity to stimulate or promote neuronal growth or regeneration.